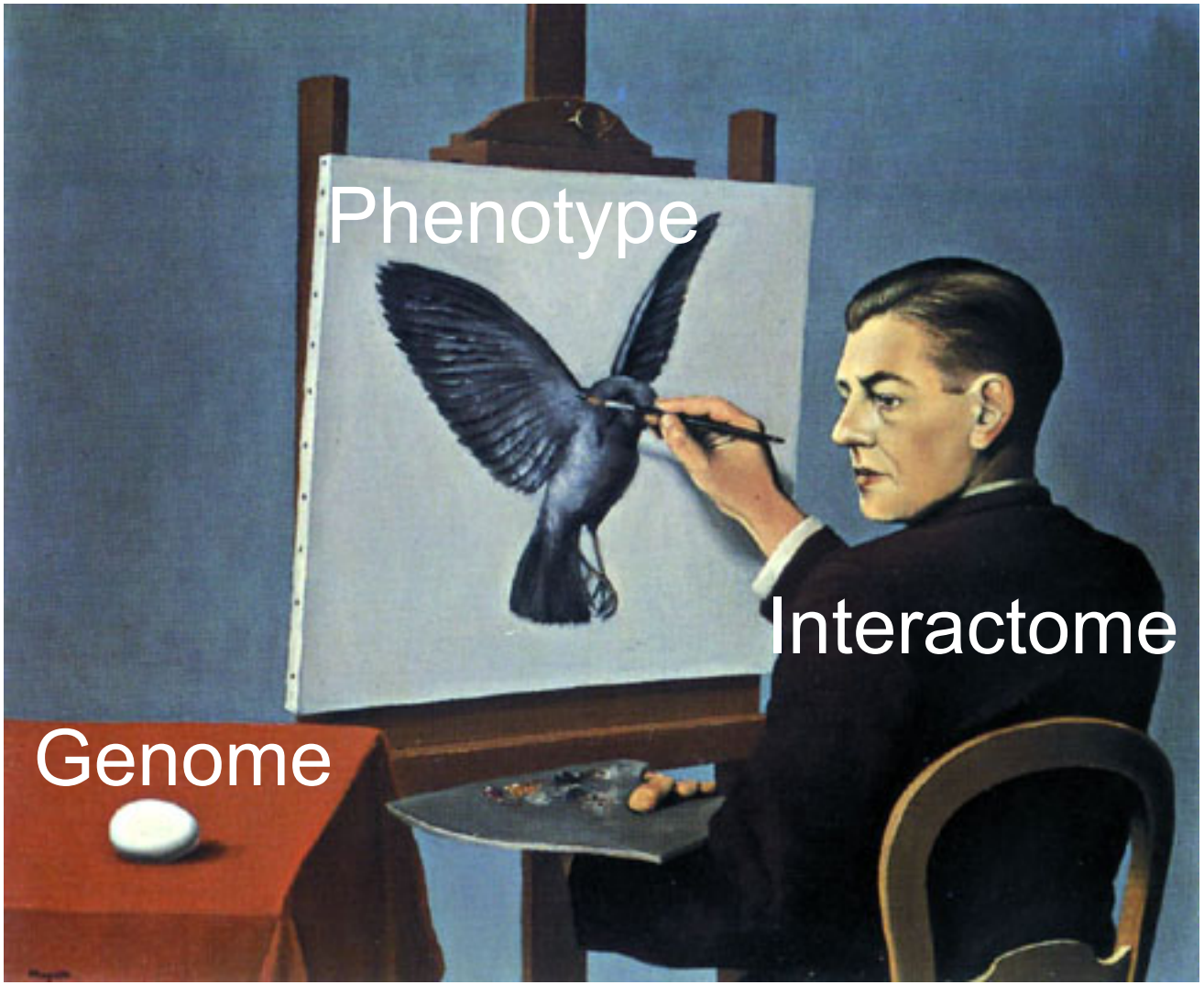
Protein – protein interactions

Interactome networks drive molecular organisation of the cell

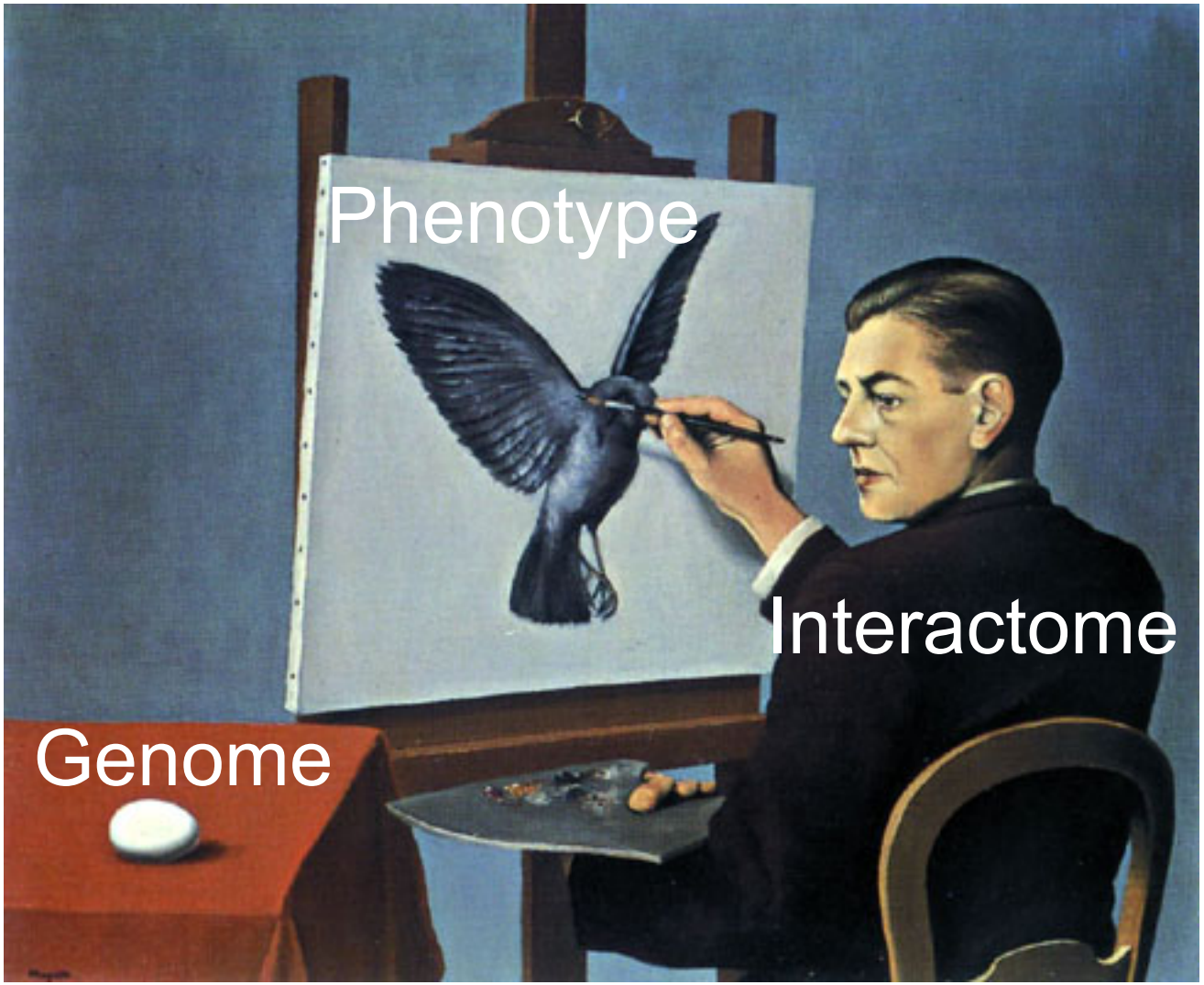
Dr. Jean-Claude Twizere

Jean-claude.twizere@ulg.ac.be

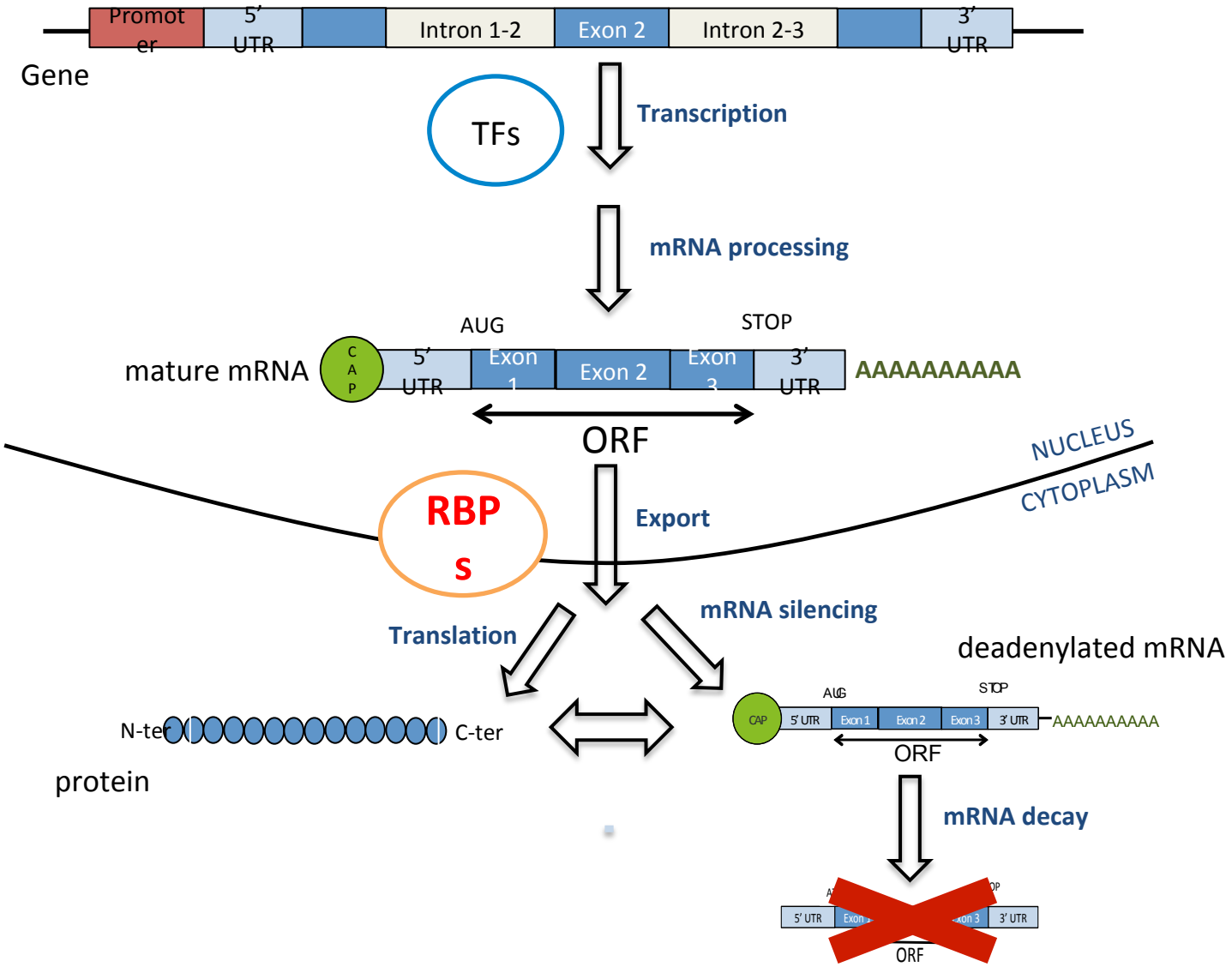

Phenotype
Interactome
Genome

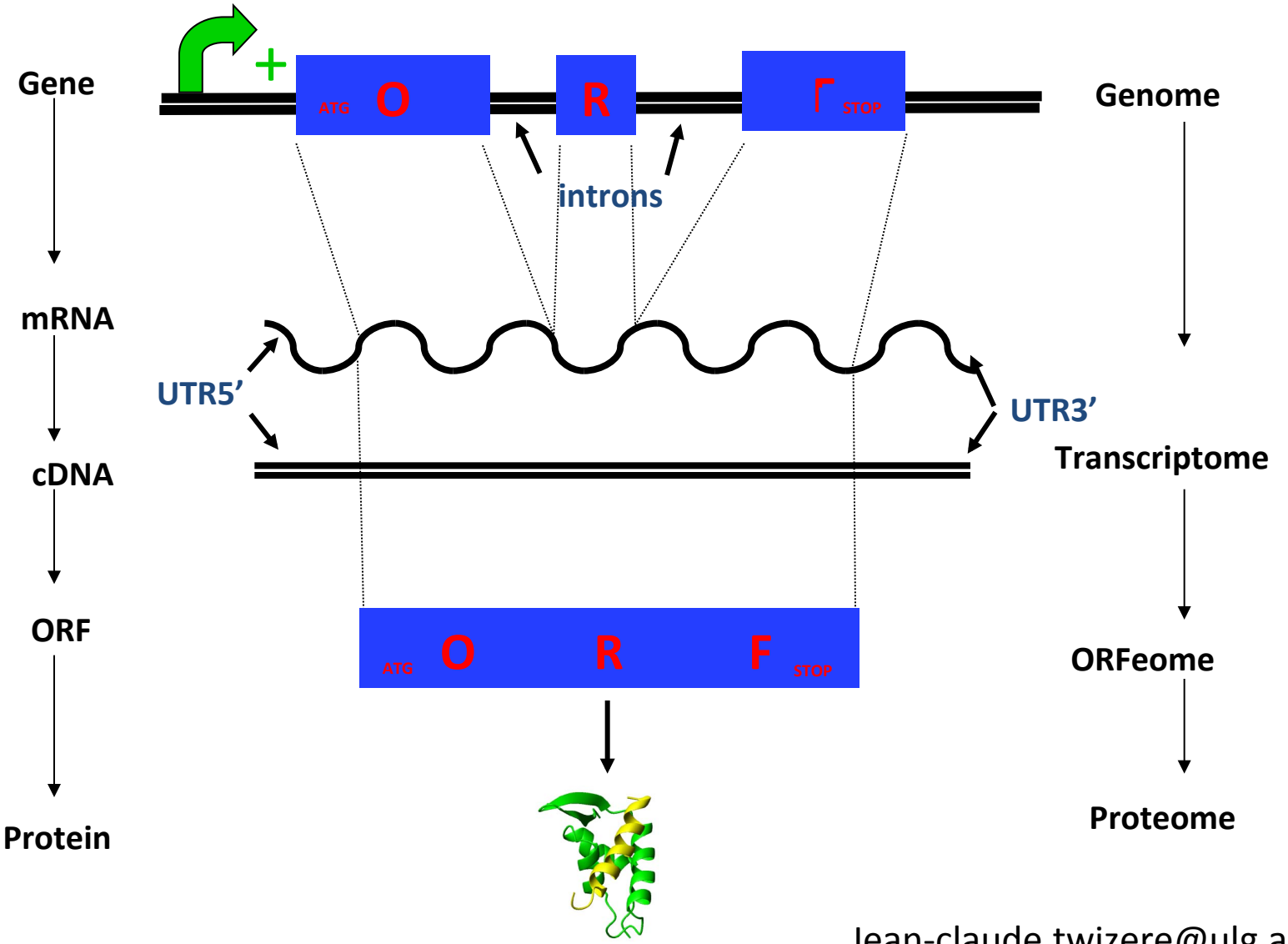
Molecular organisation of the cell

- Elucidation of Genomes, proteomes, their components and interactions
- Functional organization remains largely unknown
- Cellular function is the result of coordinated intecations
- Interaction networks essential to understand biology, disease and/or drug action


Phenotype
Interactome
Genome

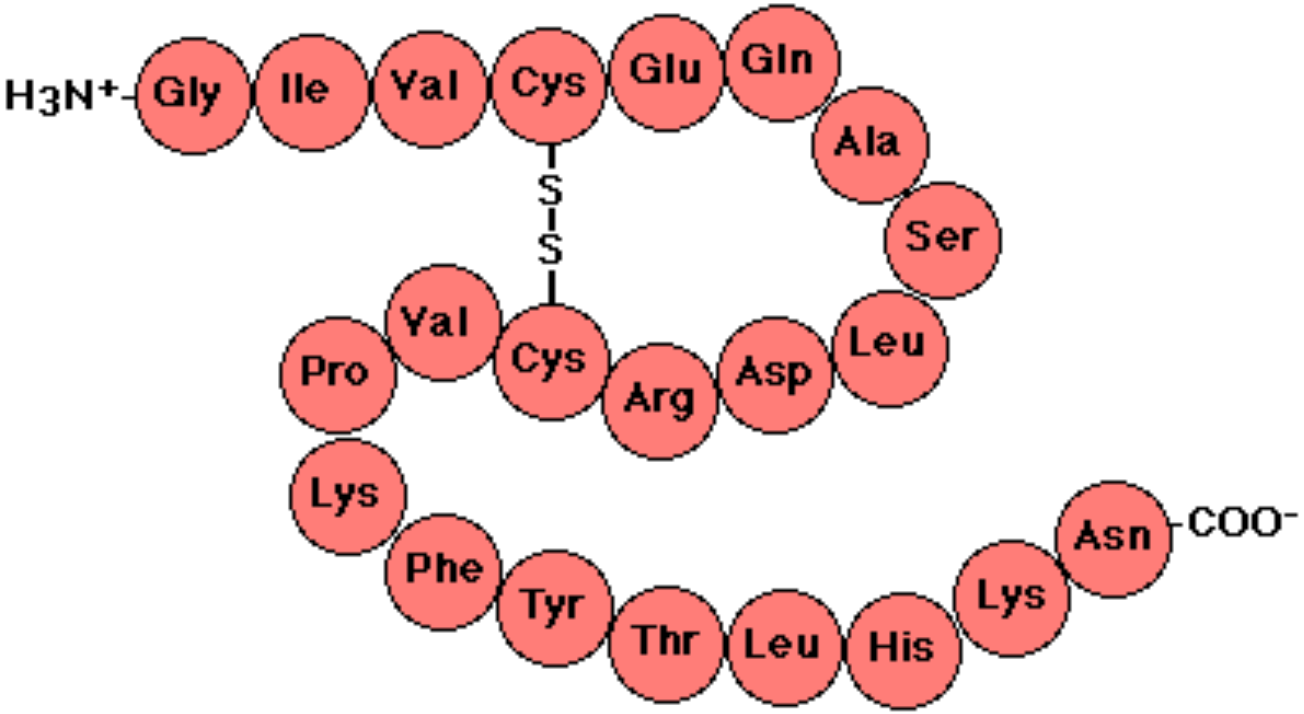
Gene expression regulation





Jean-claude.twizere@ulg.ac.be

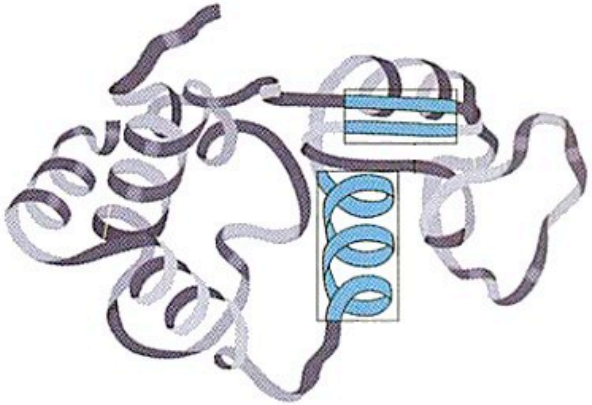
Primary structure



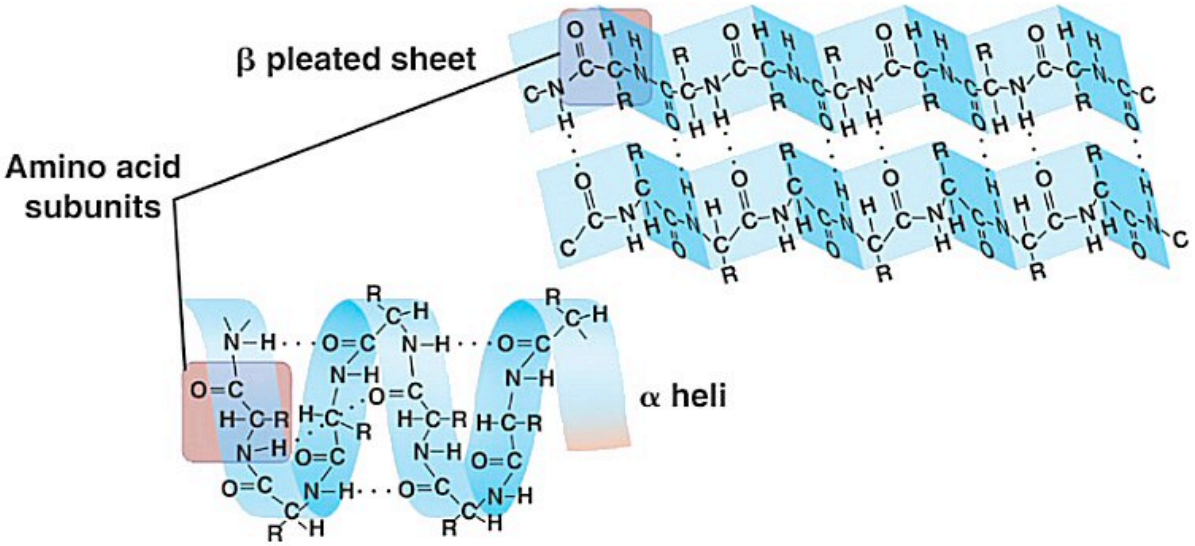
Jean-claude.twizere@ulg.ac.be

Secondary and tertiary structure

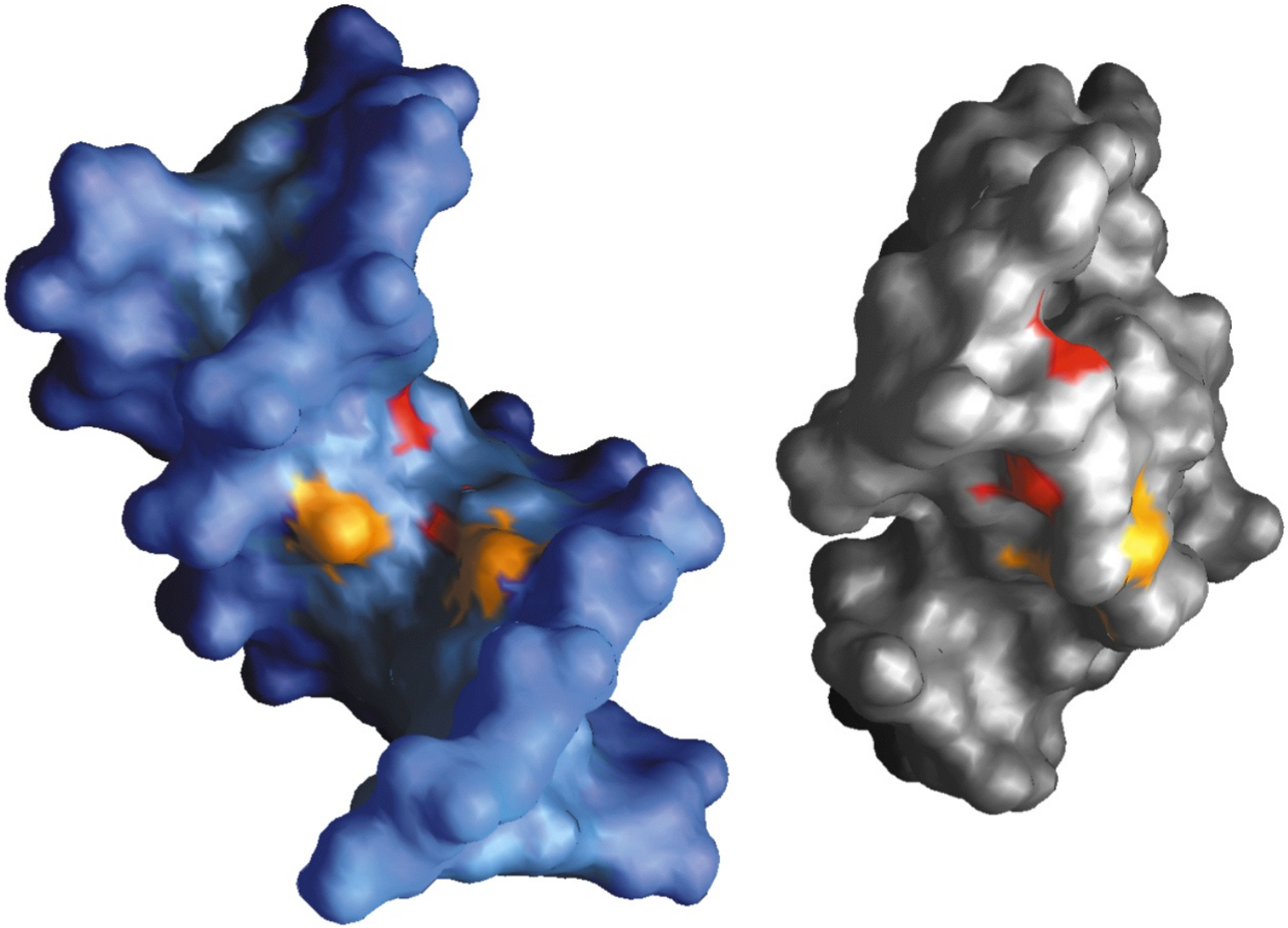
III.



II.

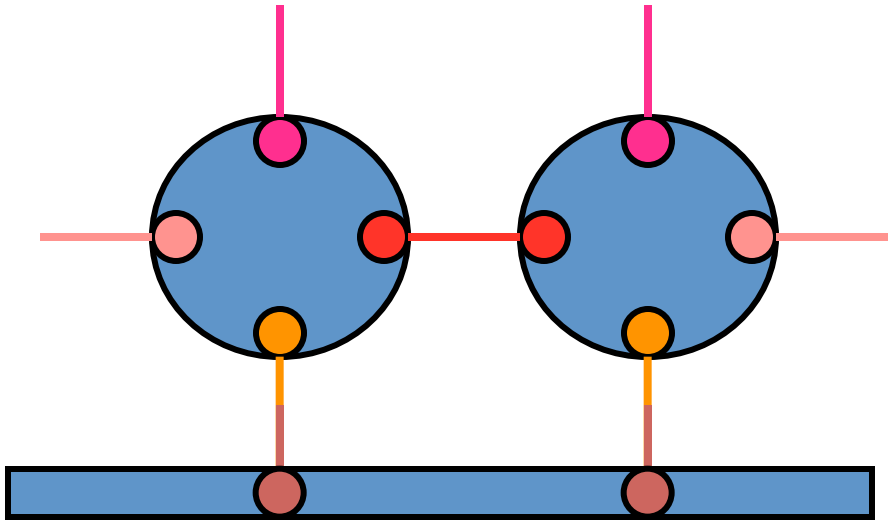


Quaternary structure



(d)

Protein Interactions

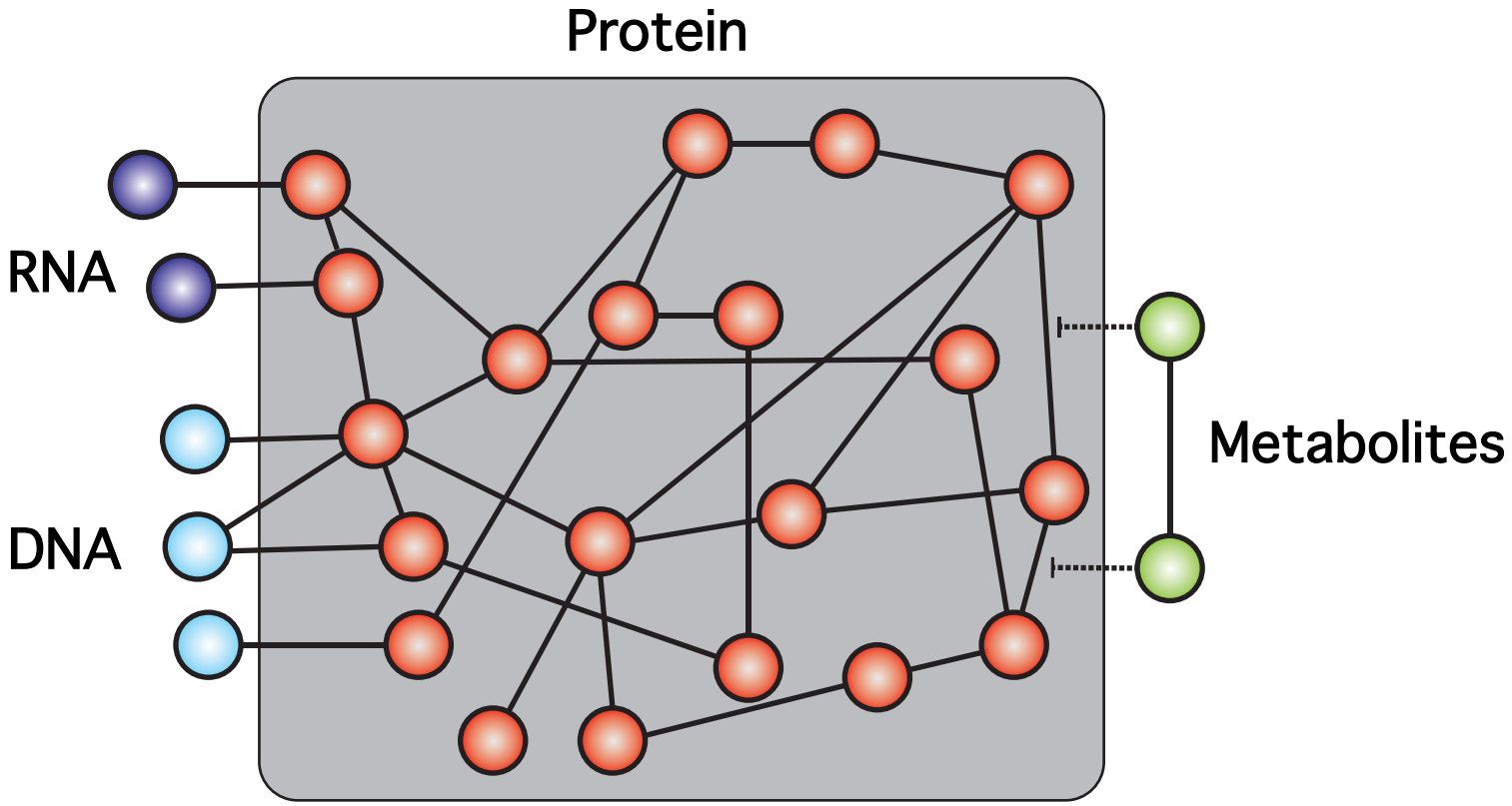


Homodimerization and DNA/protein interaction

Protein-protein interactions

- Y2H hybrid
- Affinity purification
- Energy transfer (Fluorescence = FRET)
- Co-localisation (Fluorescence based)
- Protein complementation
    - Luciferase based
    - Fluorescence based

The protein interactome network

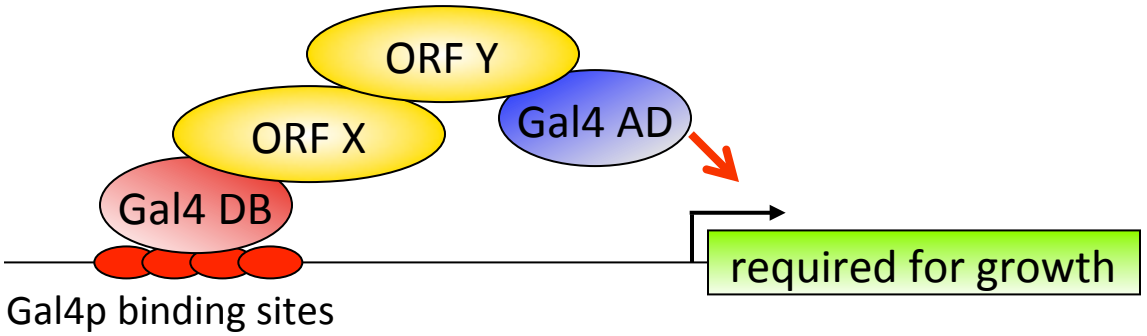


Nodes: Proteins, DNA, RNA or Metabolites
Edges: Bio-physical interactions

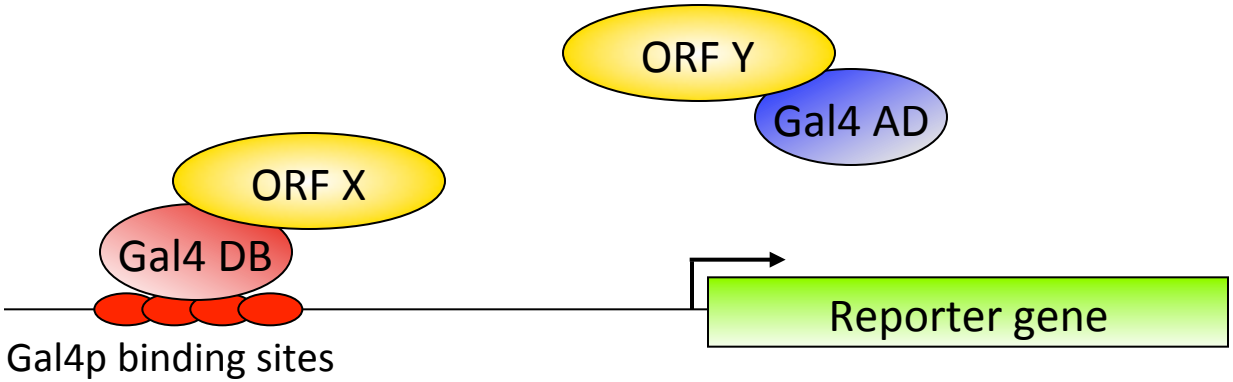
Discovering interactions: Yeast two-hybrid

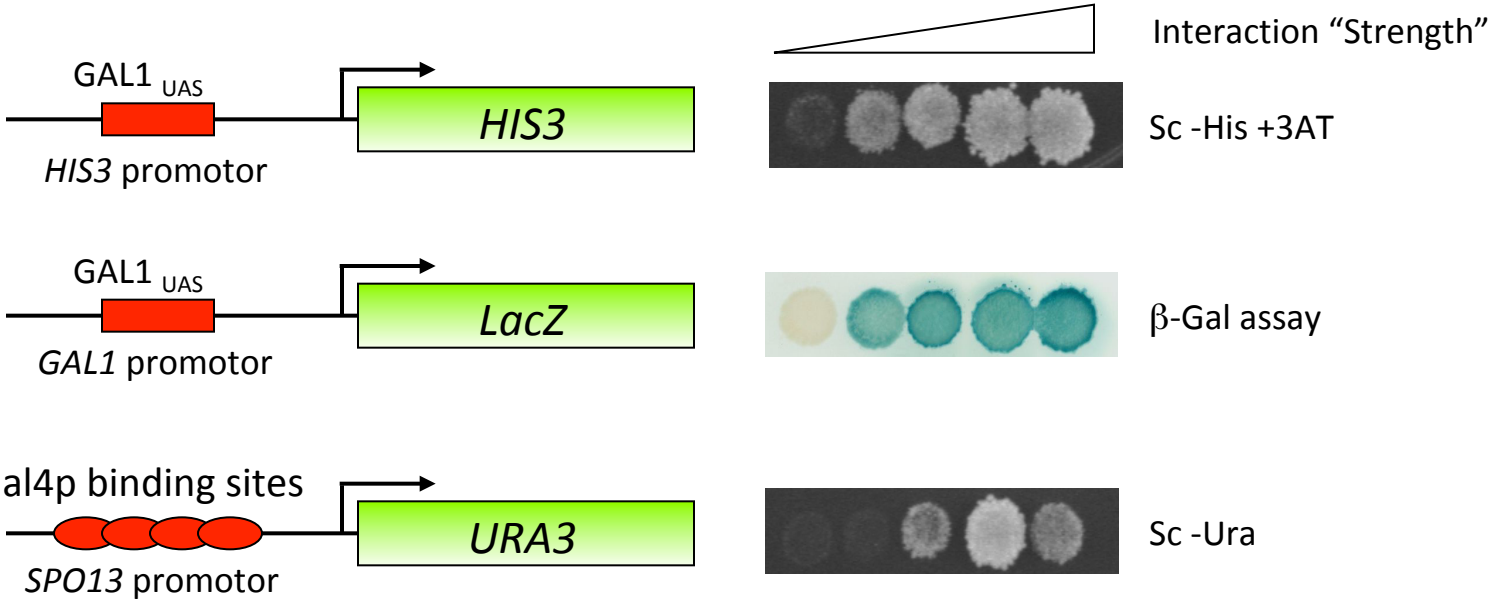
Yeast two-hybrid

- Reconstitution of GAL4 transcription factor
- Fusion proteins DB-ORFX and ORFY-AD
- Reporter gene



A positive selection of the protein – protein interactions





Yeast two-hybrid

Reagents (retroviruses side)

genes + fragments

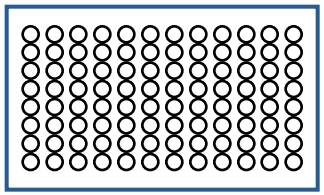
Gateway cloning

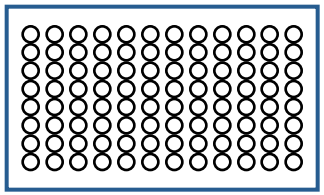
DB, AD expression vectors

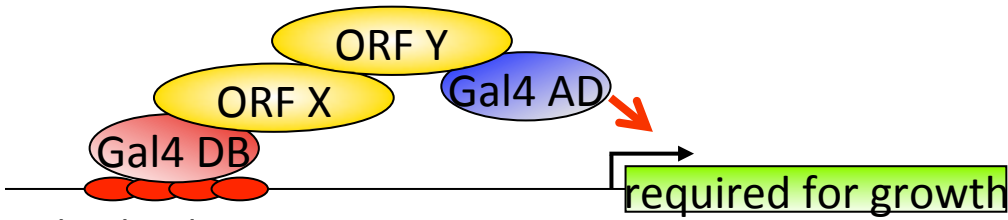
Yeast transformation

Mat a (Y8900 or Mav103)

Mat a (Y8800 or Mav203)

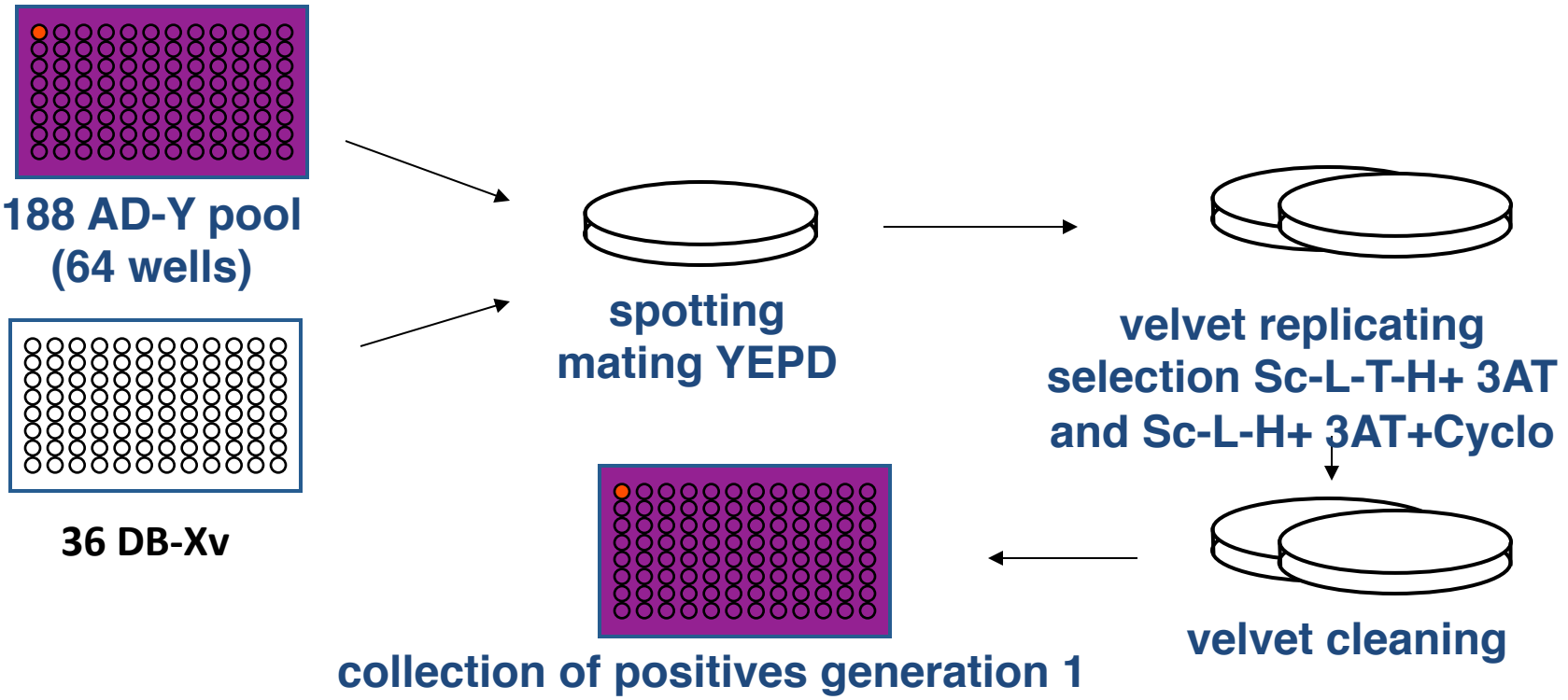


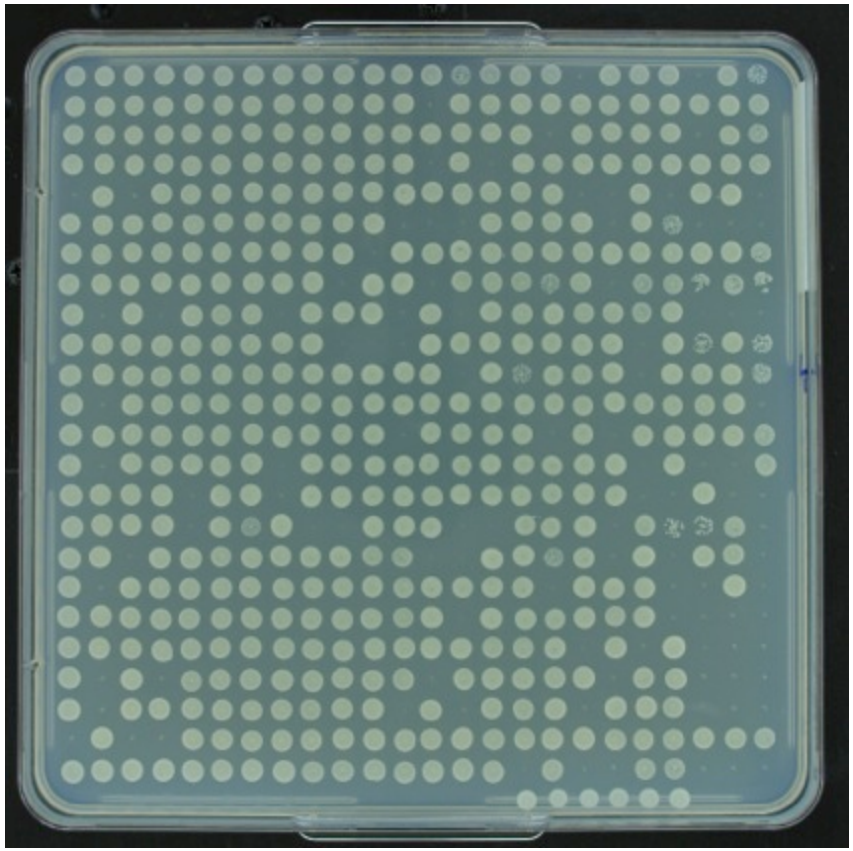




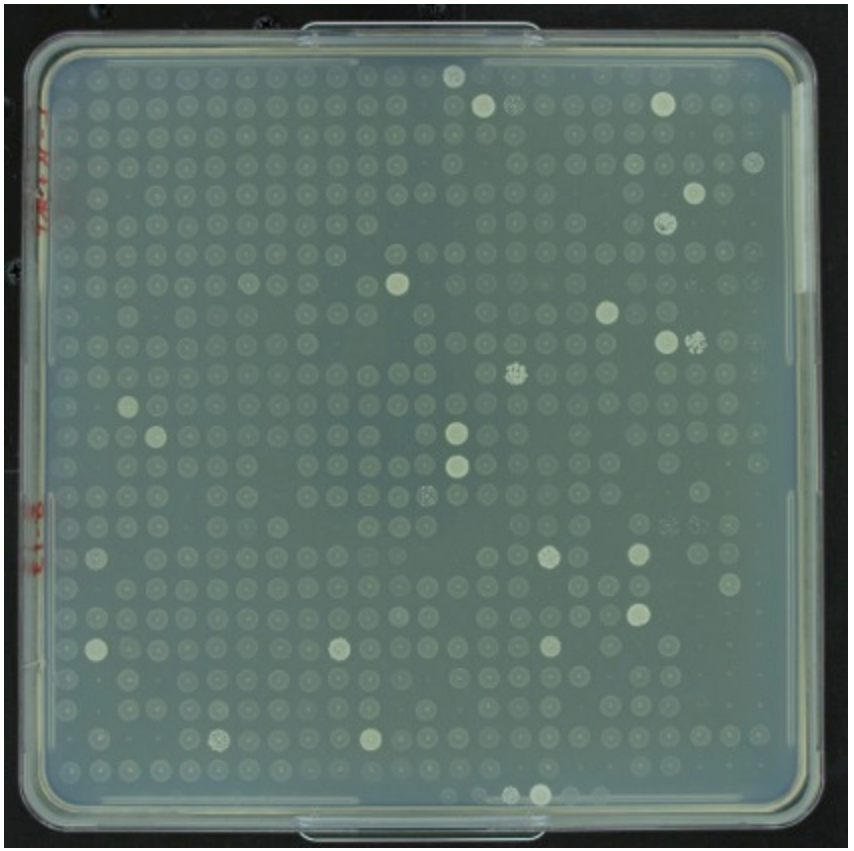
Gal4p binding sites

High-throughput Y2H mating



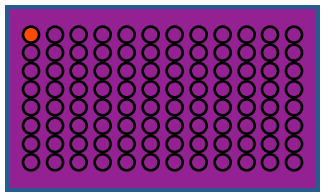


SC-LT

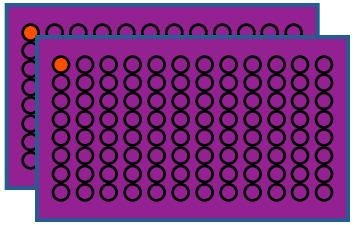


SC-LTH +1 mM 3AT

Sequencing



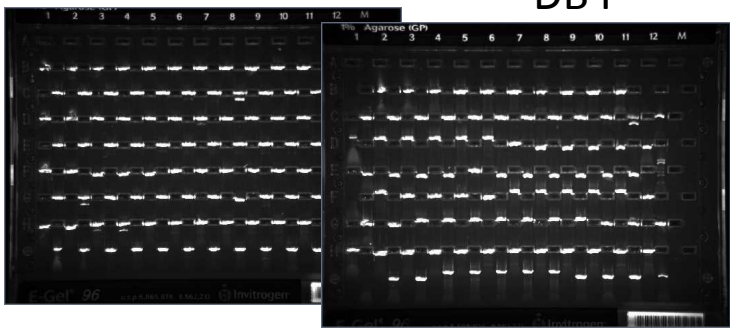
positives
generation 2



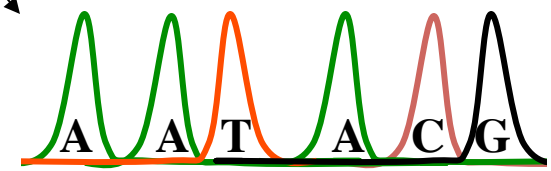
PCR
AD and DB

AD I

DB I



electrophoresis gel

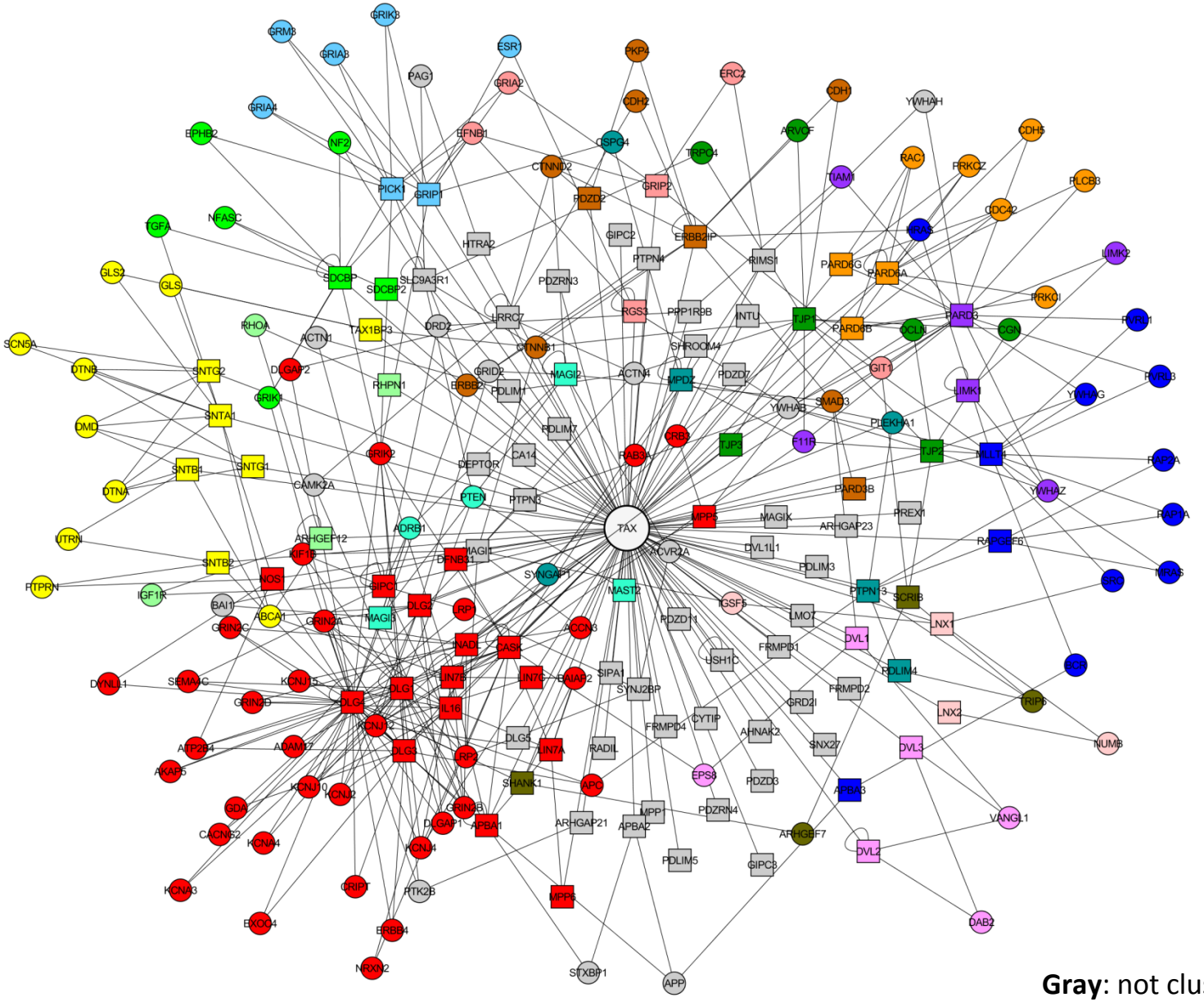


sequencing at Agencourt

protein interaction map

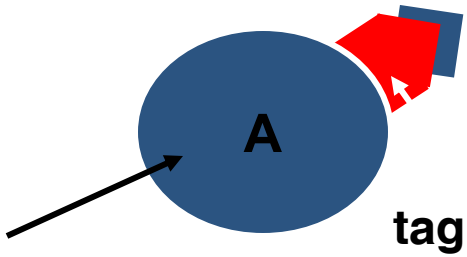
bioinformatic
analysis

TAX / PDZ PROTEINS INTERACTOME



Gray: not clustered
Other color: clustered

Affinity purification/mass spectrometry

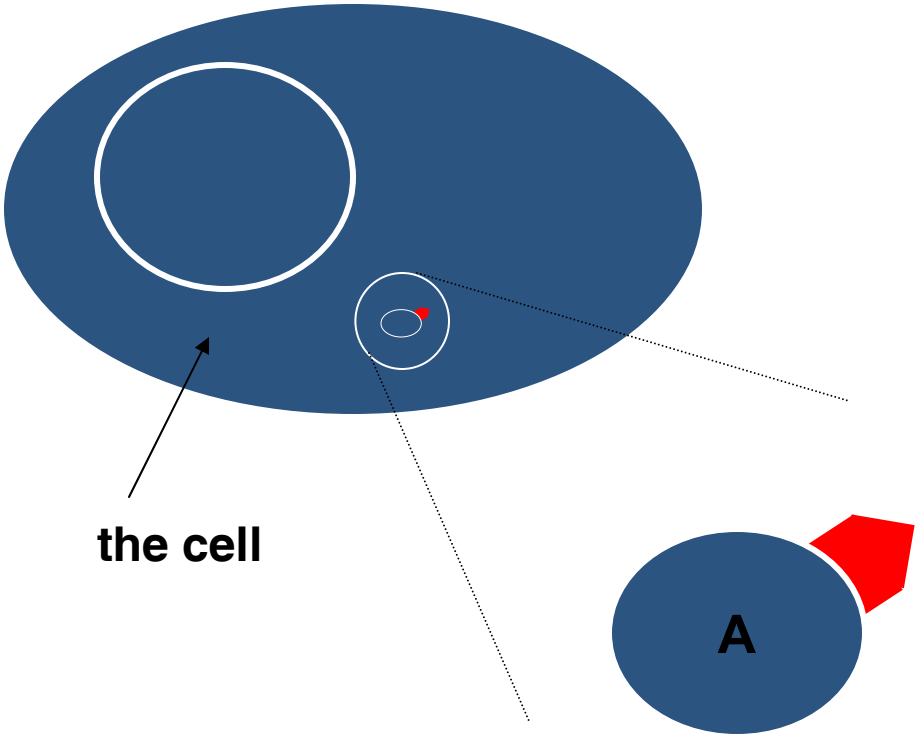


this molecule will bind the 'tag'.

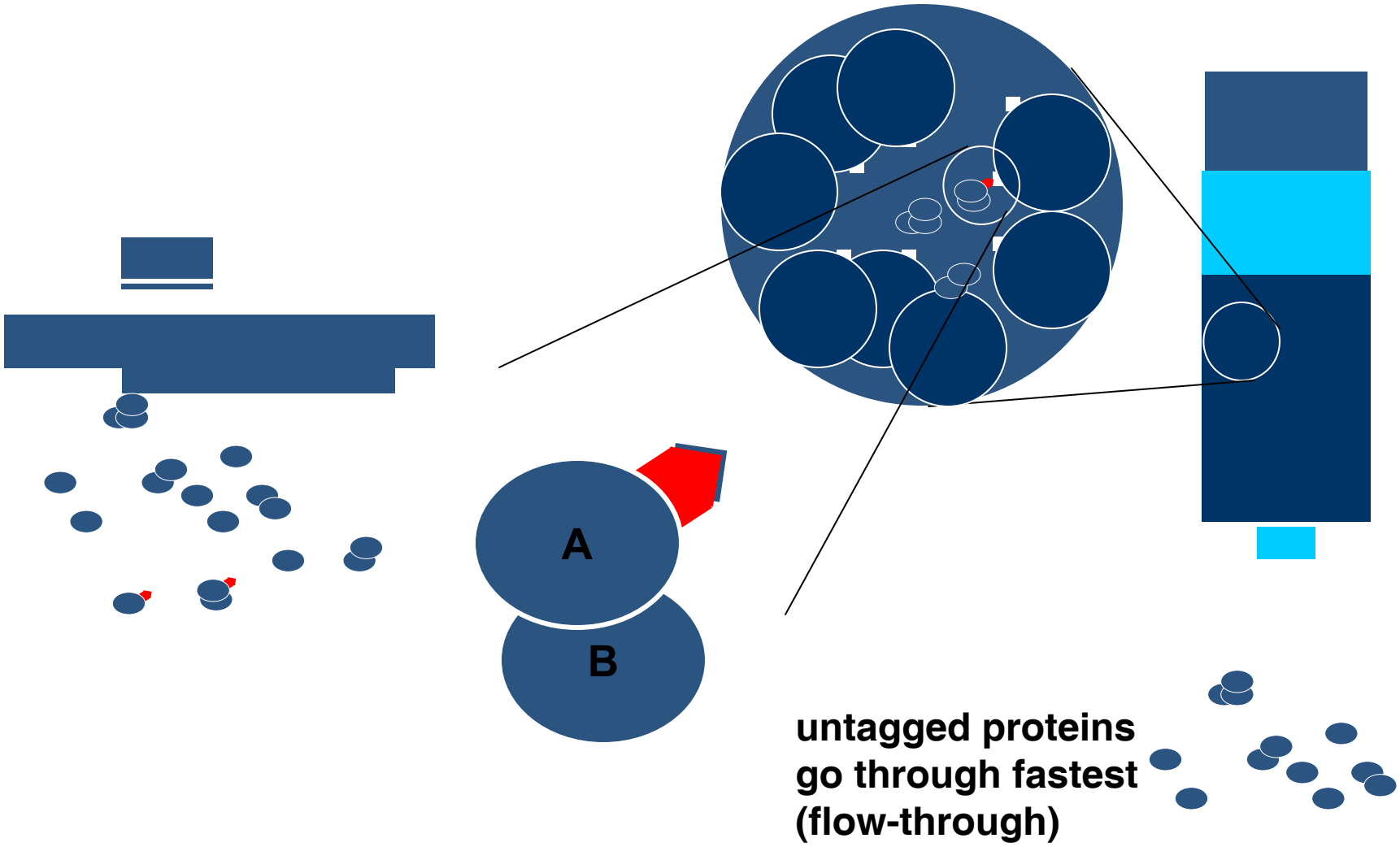
tag modification
(e.g. HA/GST/His, FLAG, MYC, TAP)

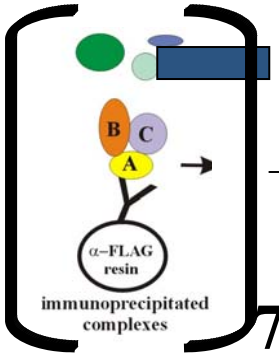
Protein of interest

Affinity purification/mass spectrometry

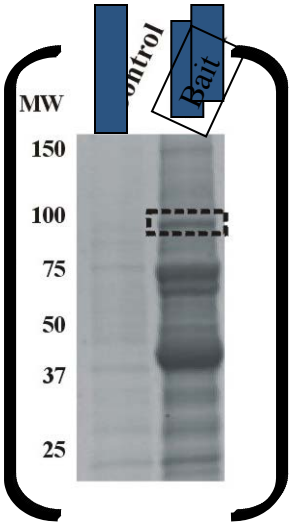


Affinity purification/mass spectrometry

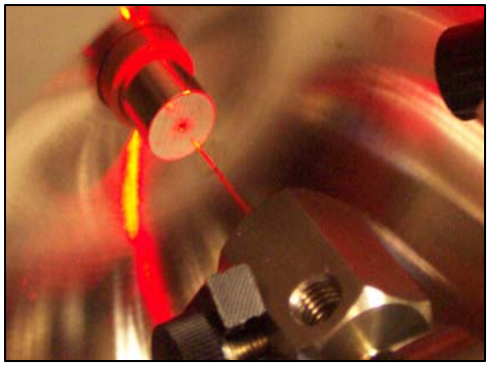




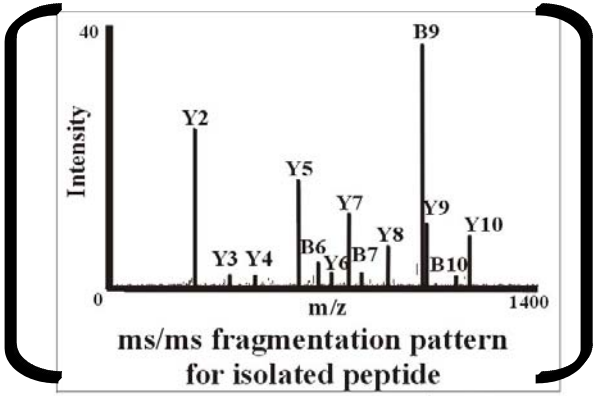
725



1,558

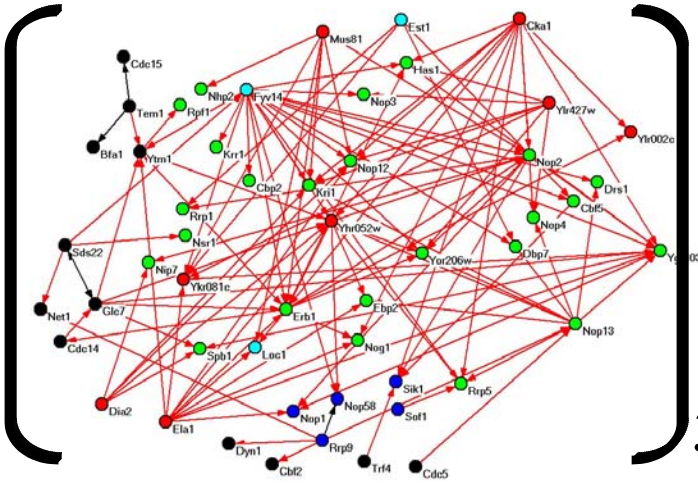


HTP-MS/MS Network



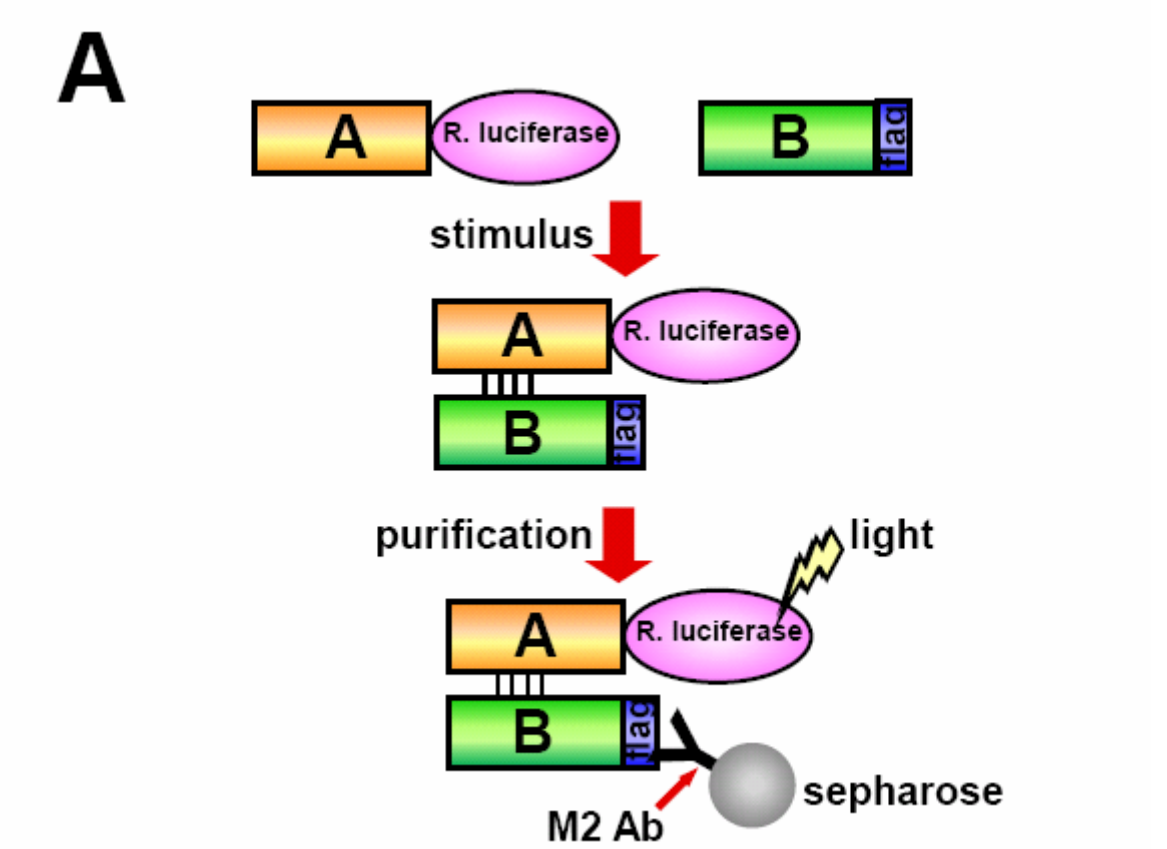
950,000

Database searching of fragmentation patterns



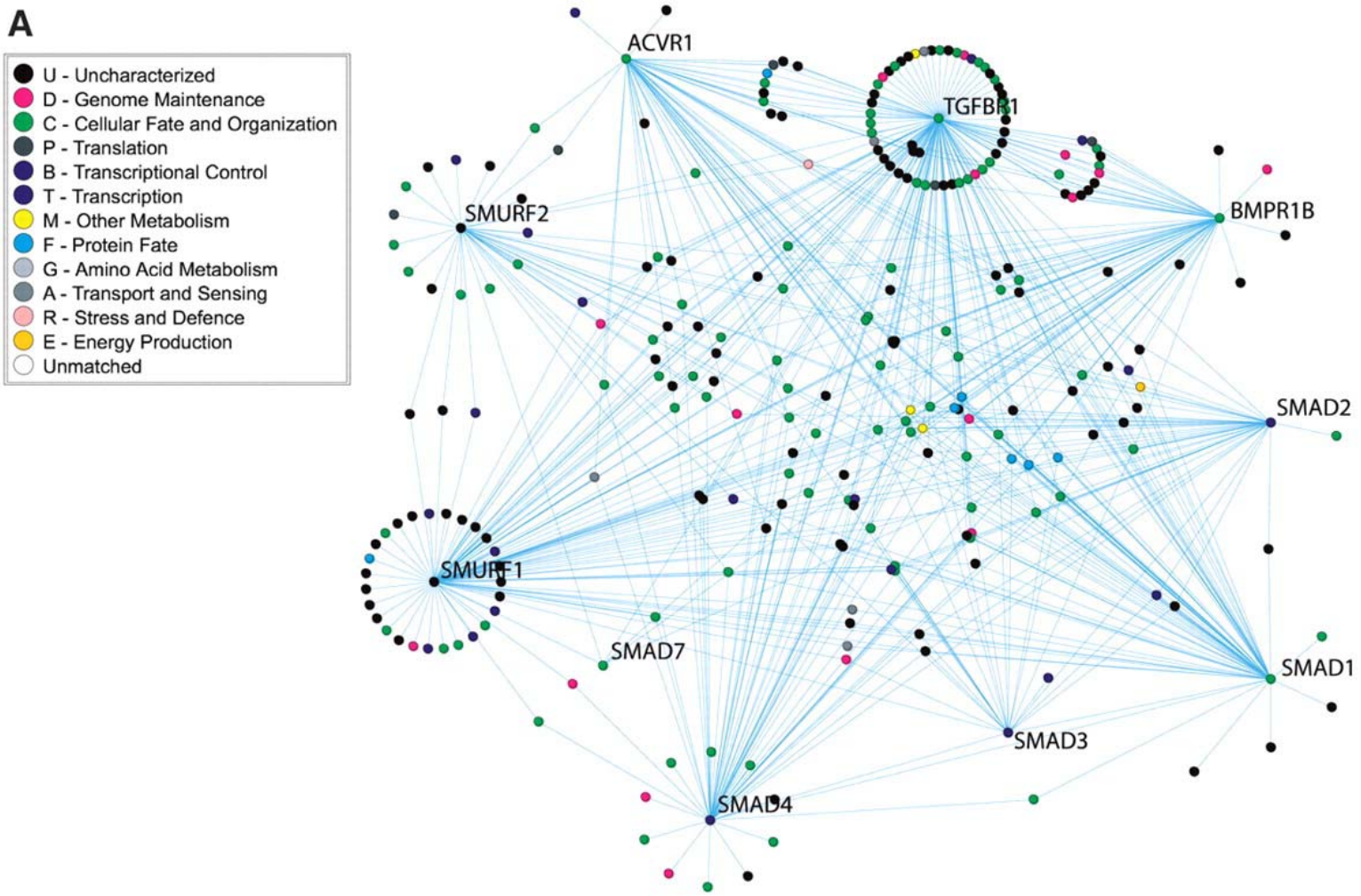
3,617

LUminescence-based Mammalian IntERactome mapping (LUMIER)

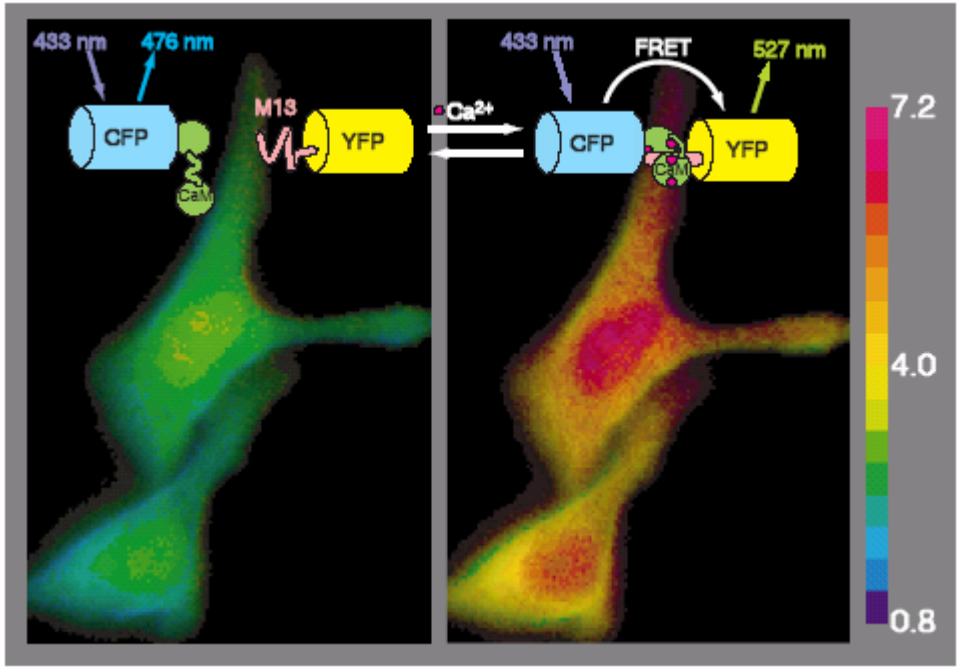


Barrios-Rodiles M, et al.
High-throughput mapping of a dynamic signaling network in mammalian cells.
Science. 2005 Mar 11;307(5715):1621-5.

High-throughput screening in 293 cells using the Lumier approach



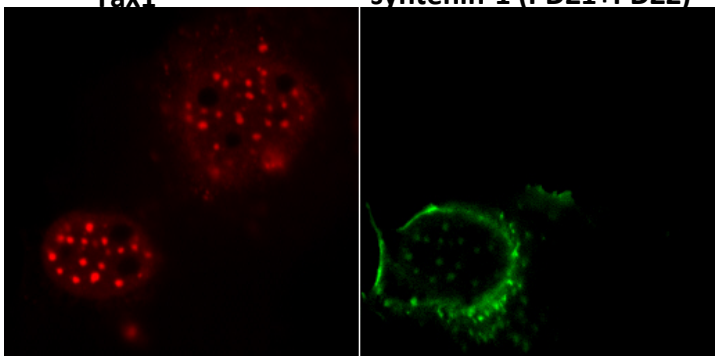
Monitoring assembly: FRET



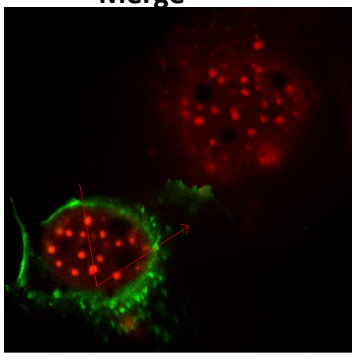
Monitoring interactions: co-localization

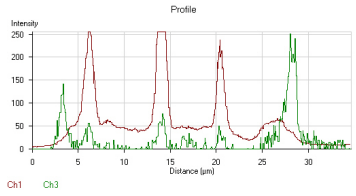
Tax1

syntenin-1 (PDZ1+PDZ2)



Merge

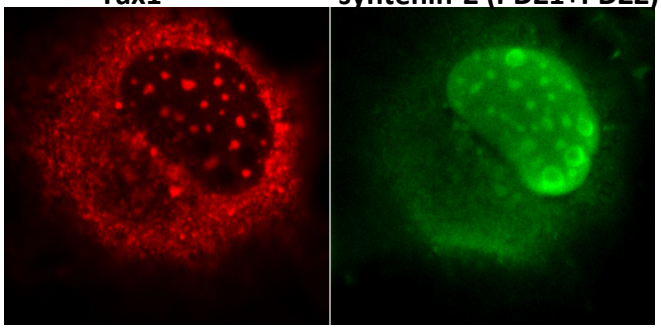




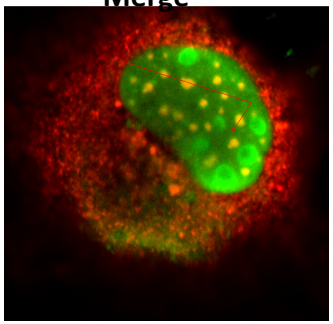
No colocalization between syntenin-1 (PDZ1+PDZ2) and Tax1

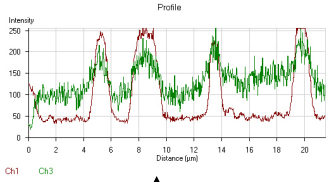
Tax1

syntenin-2 (PDZ1+PDZ2)



Merge

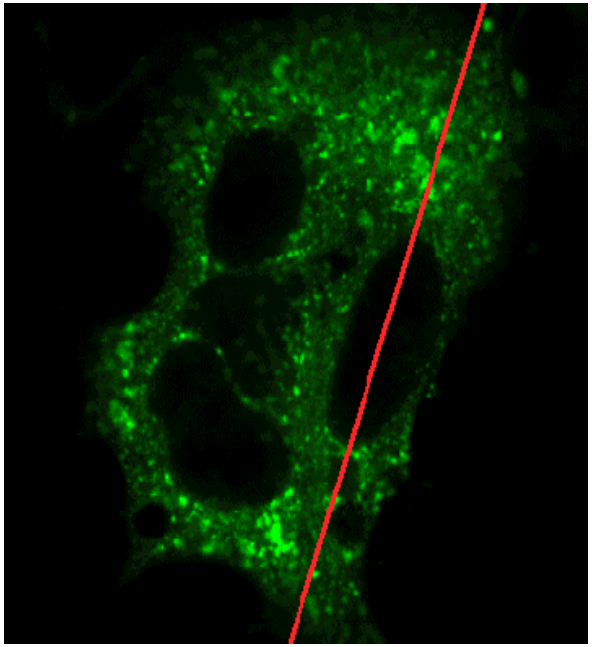




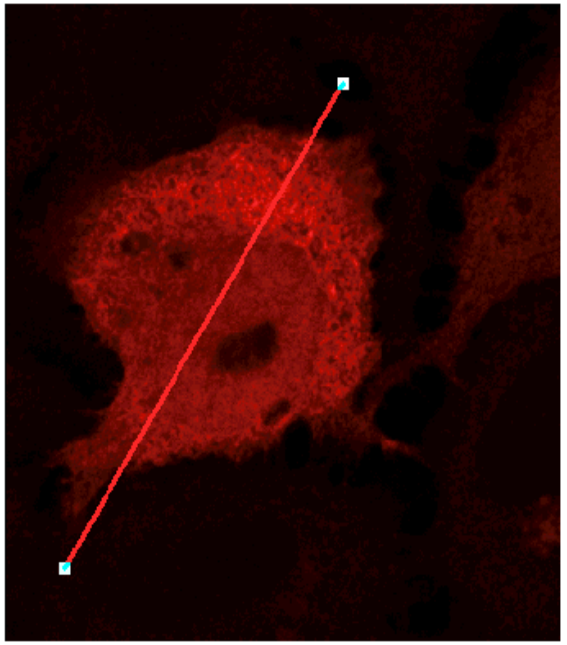
colocalization between syntenin-2 (PDZ1+PDZ2) and Tax1

Monitoring interactions: localization change

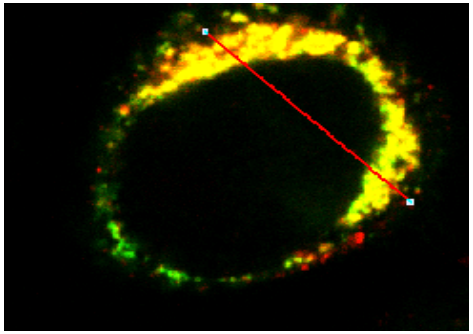
GFPTTP

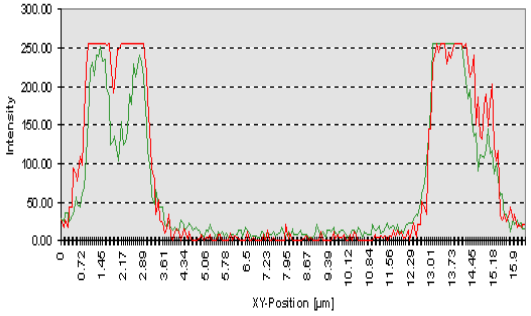


Tax



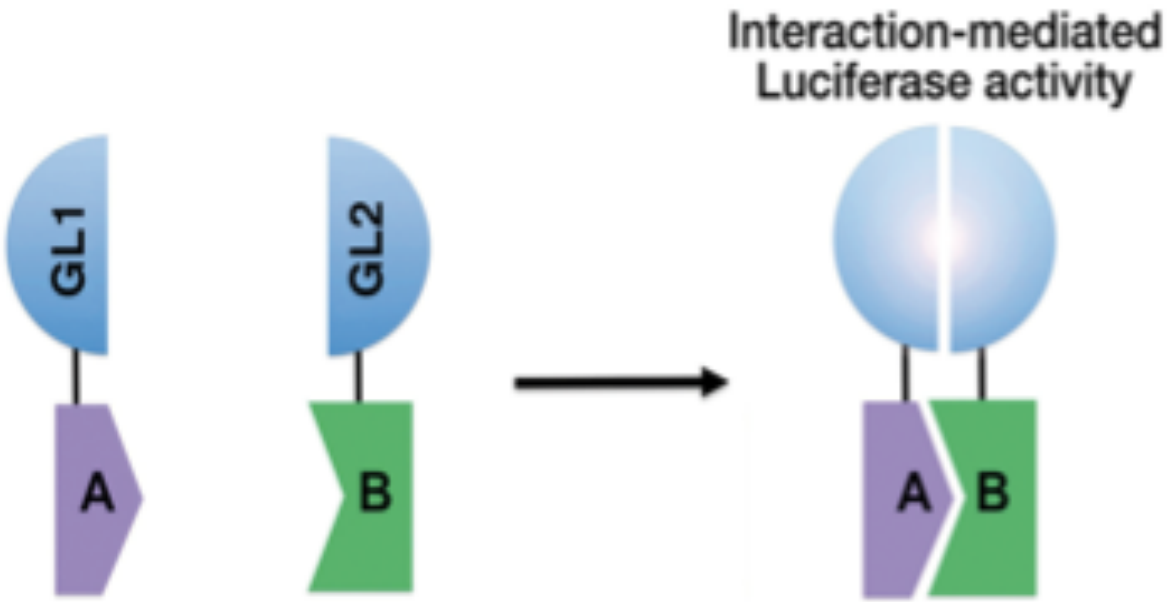
Tax1 et TTP



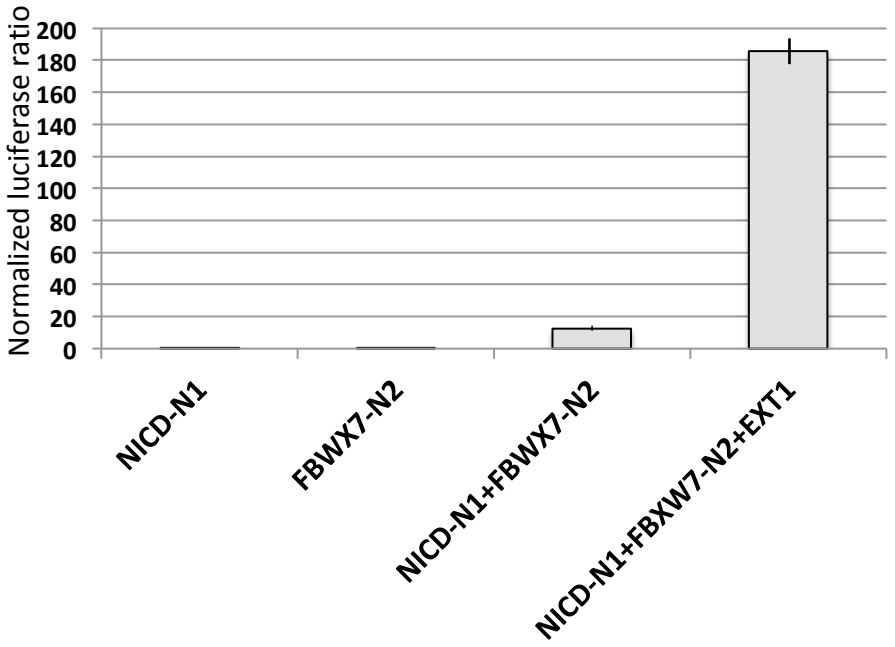


Monitoring interactions: protein complementation

Gaussia princeps luciferase (GL)-based protein complementation assay (PCA)



Protein complementation assay



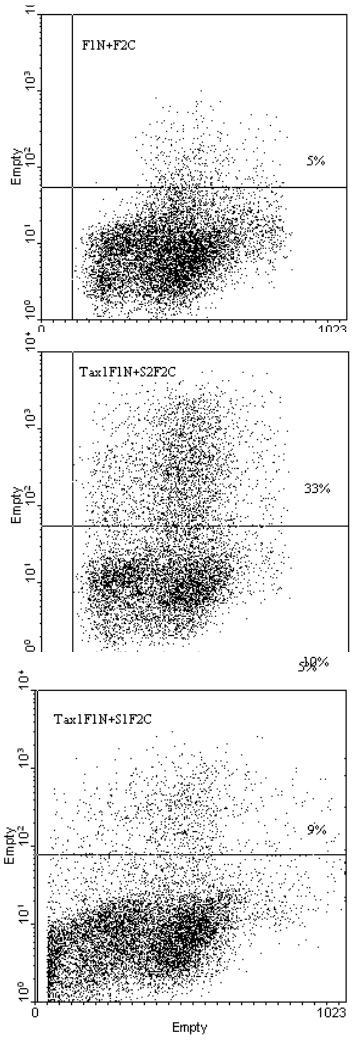
Monitoring interactions: protein complementation

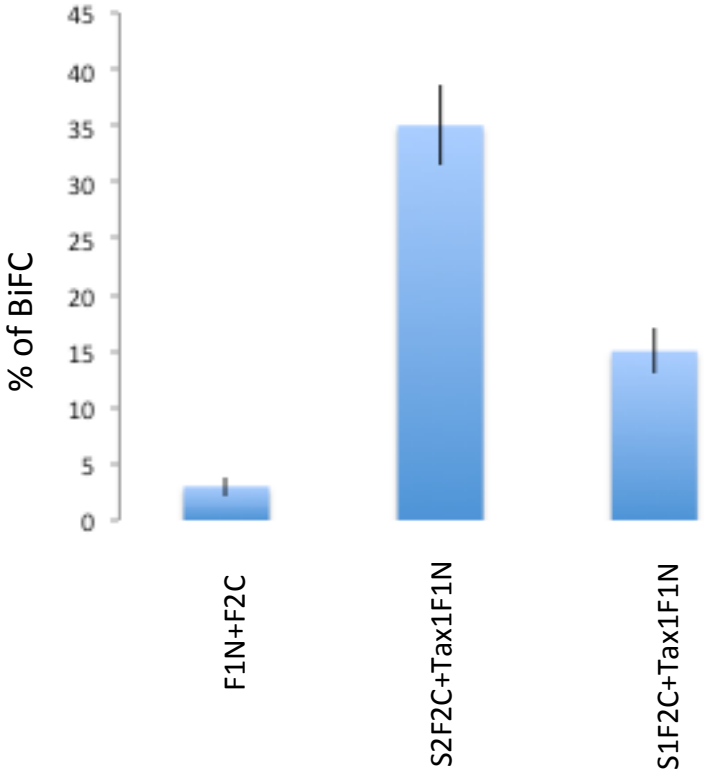
Bi-molecular fluorescence complementation

Control

Syntenin2 +Tax1

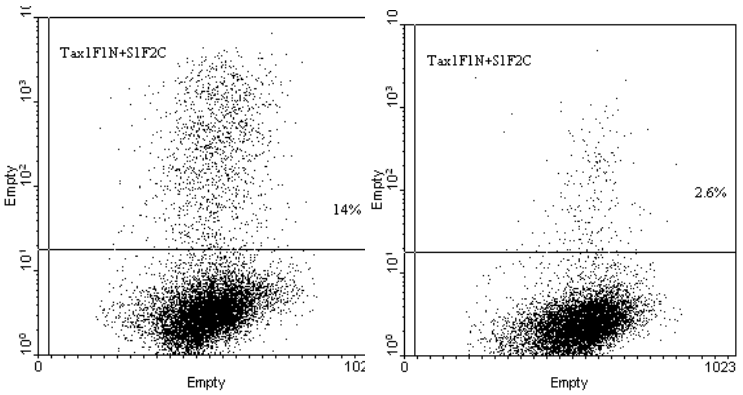
Syntenin1 +Tax1



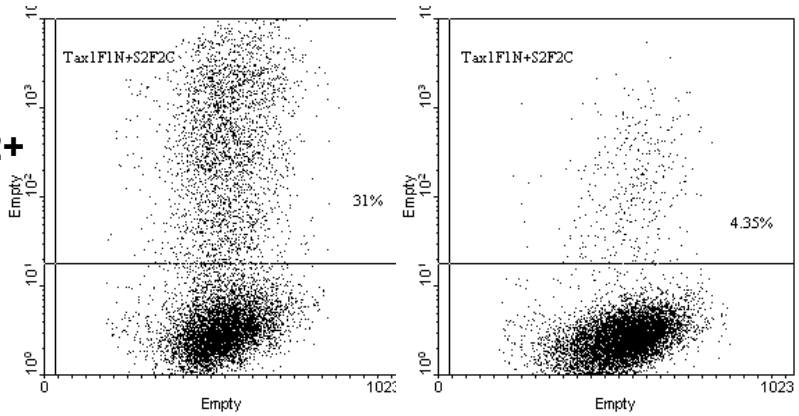


FJ9 inhibits Tax/syntenin interaction

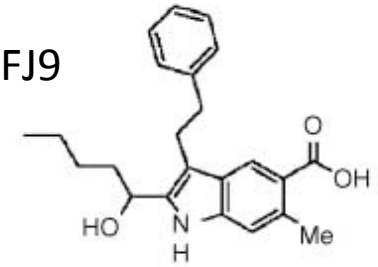
Syntenin1+ Tax1

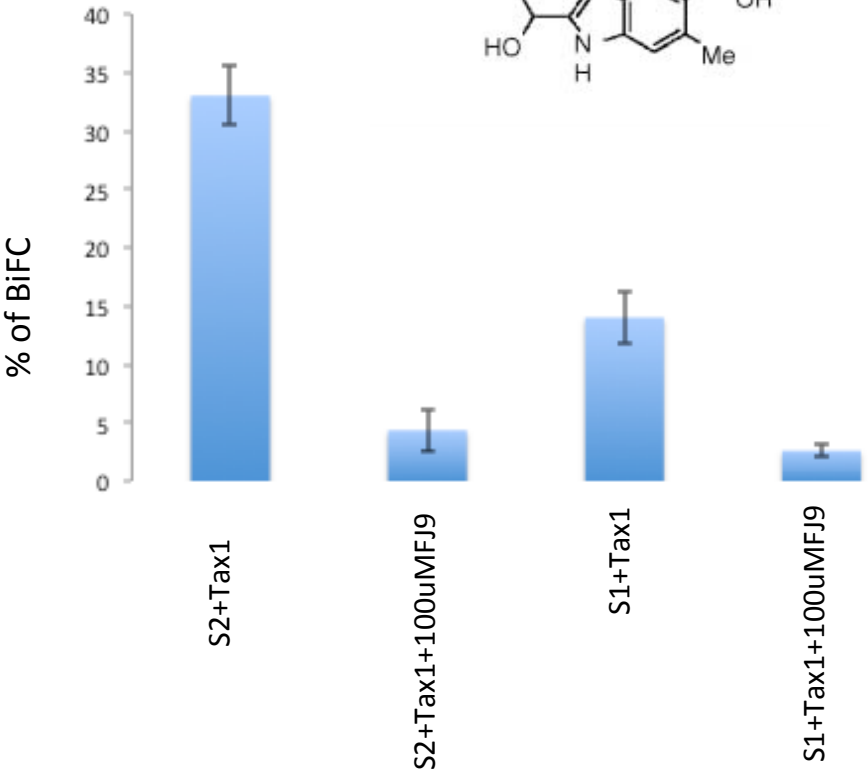


Syntenin2+ Tax1



FJ9

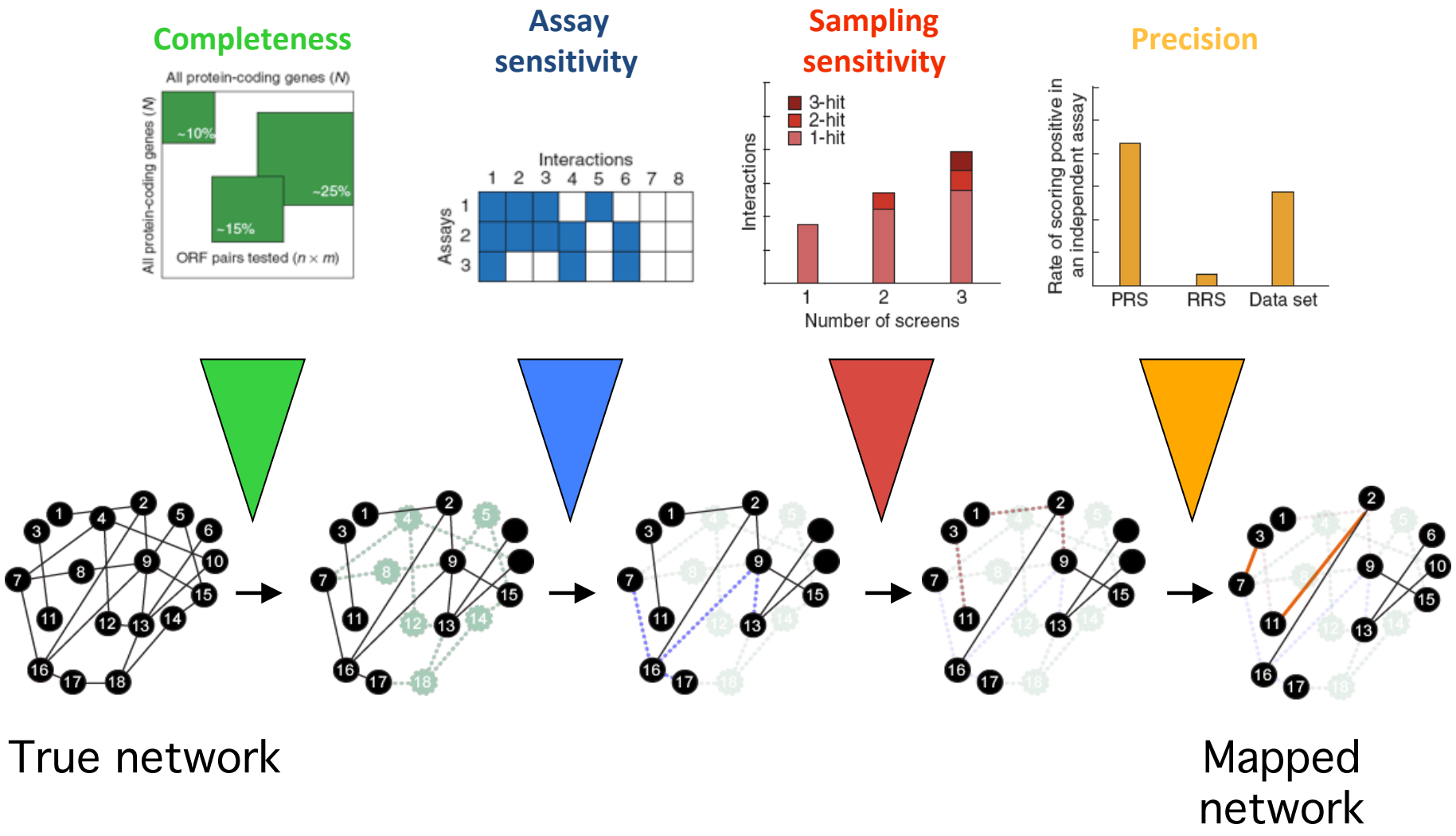




Protein-protein interactions

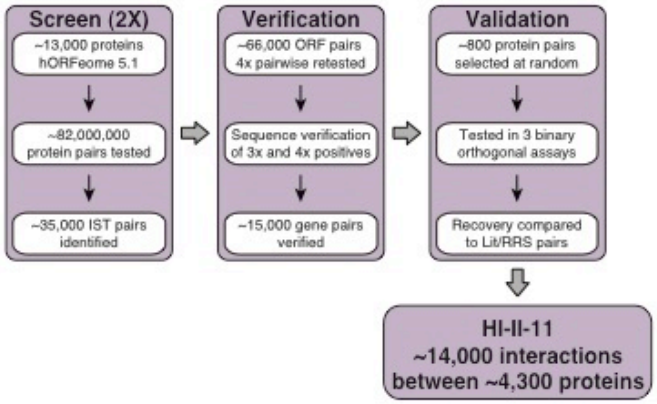
- Affinity purification
- Y2H hybrid
- Energy transfer (Fluorescence = FRET)
- Co-localisation (Fluorescence based)
- Protein complementation
    - Luciferase based
    - Fluorescence based

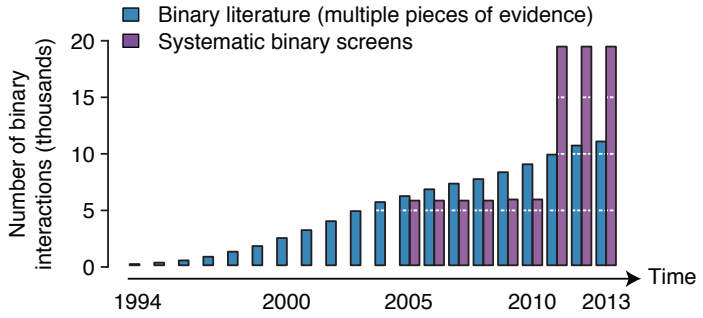
Empirical framework

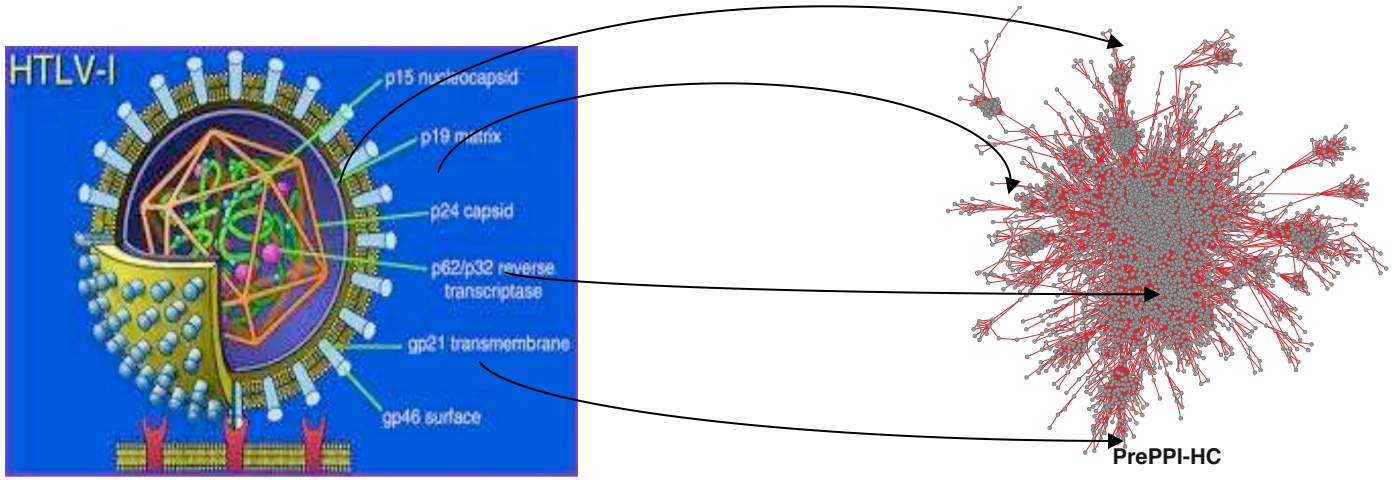


Adapted from Venkatesan, K. *et al.* Nat Meth (2009)

Interactomes mapping applications

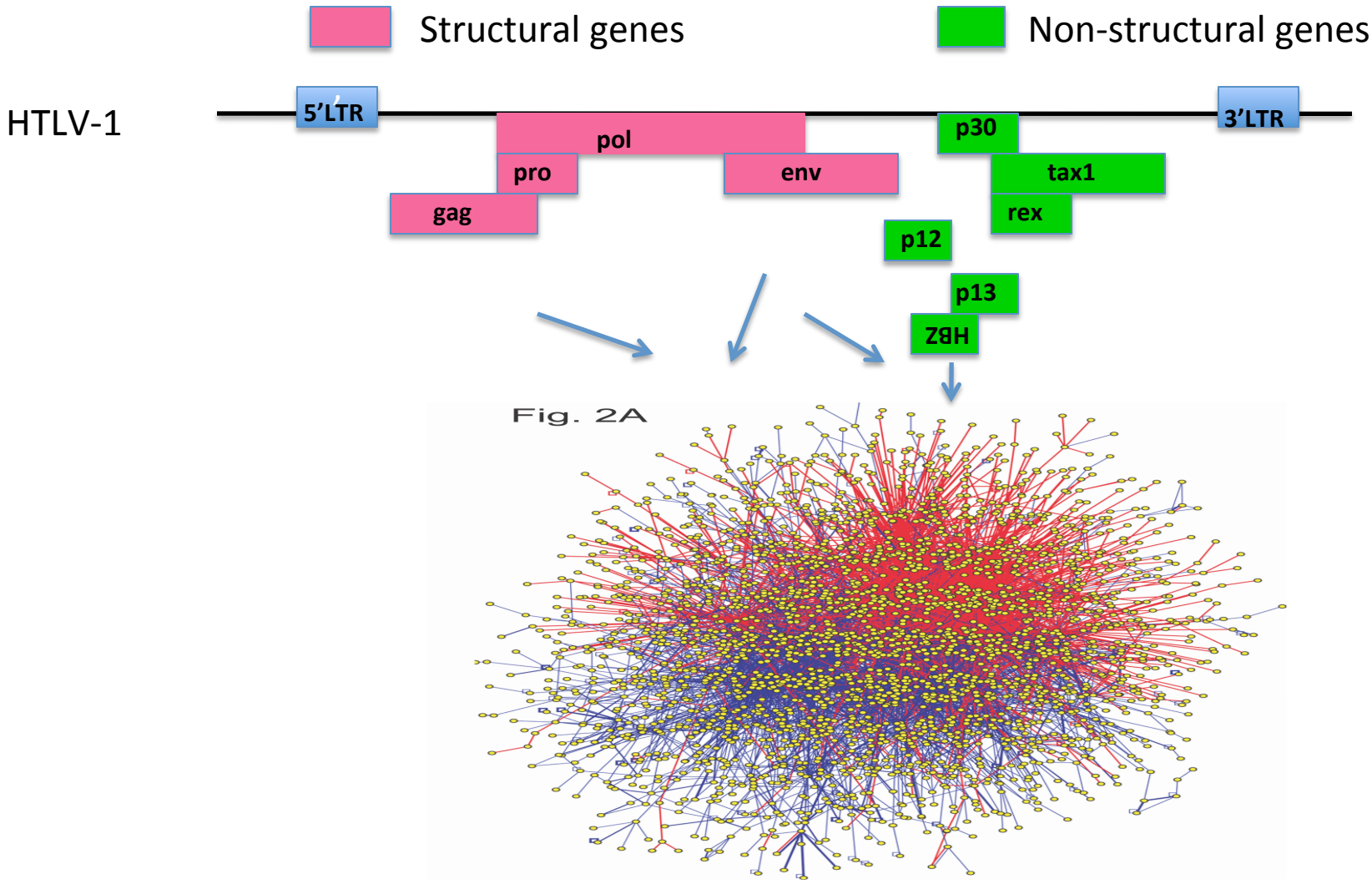






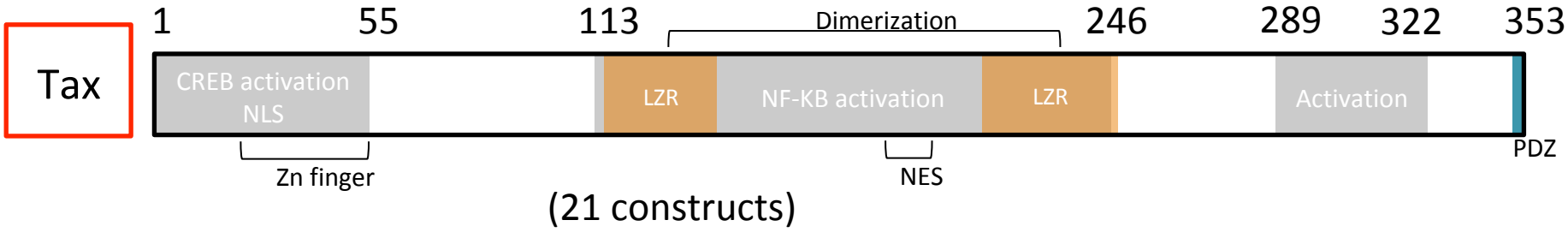
jean-claude.twizere@ulg.ac.be

Host – Pathogens interactome

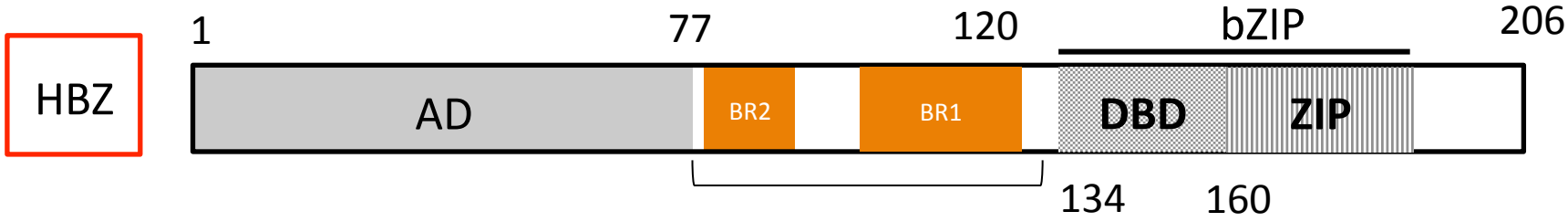


Preliminary results

Cloning of Tax and HBZ constructs



(21 constructs)



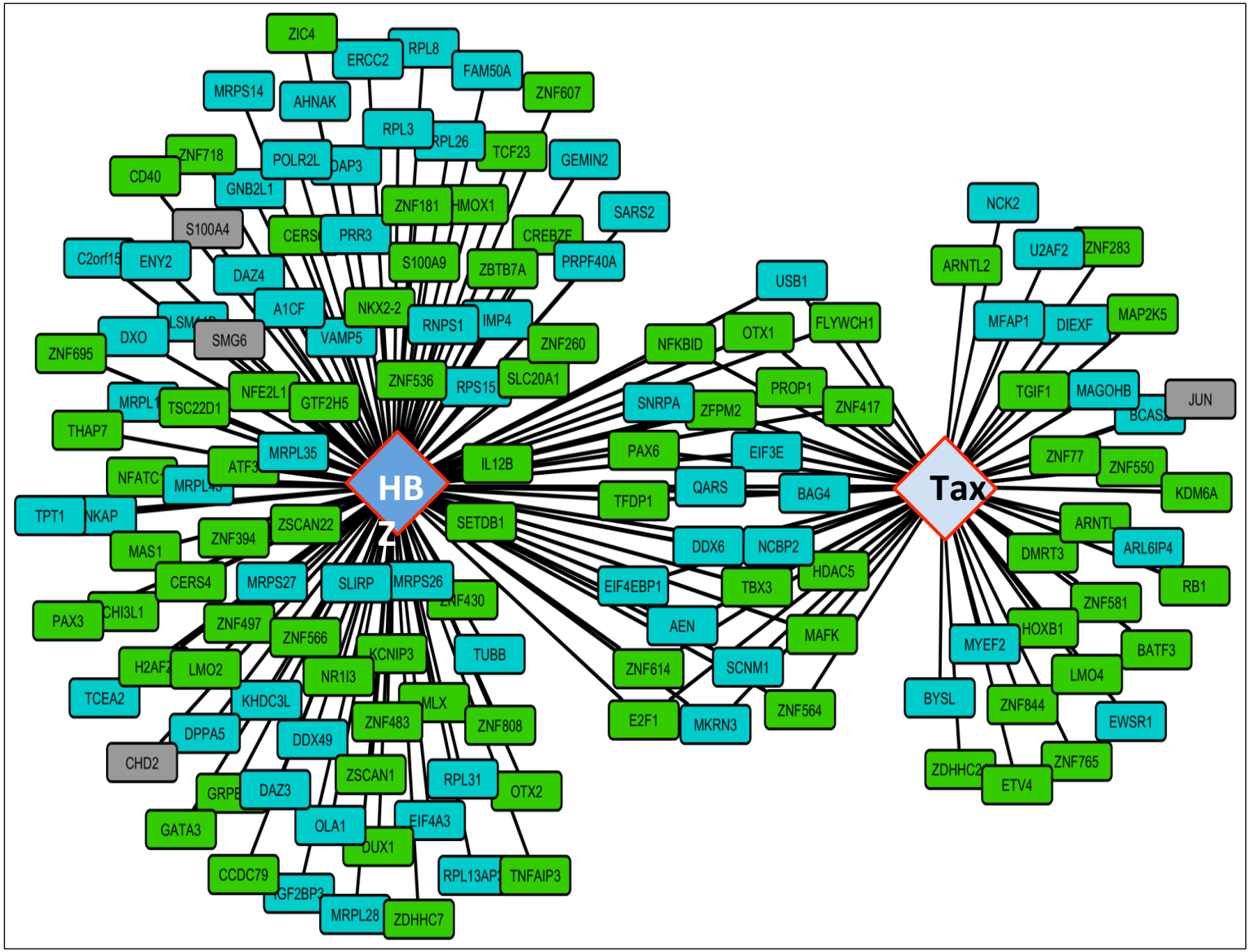
(9 constructs)

Comprehensive mapping of Tax/HBZ interactome with Transcriptional and Post-transcriptional regulators

RBPs

TFs

RBPs/ TFs

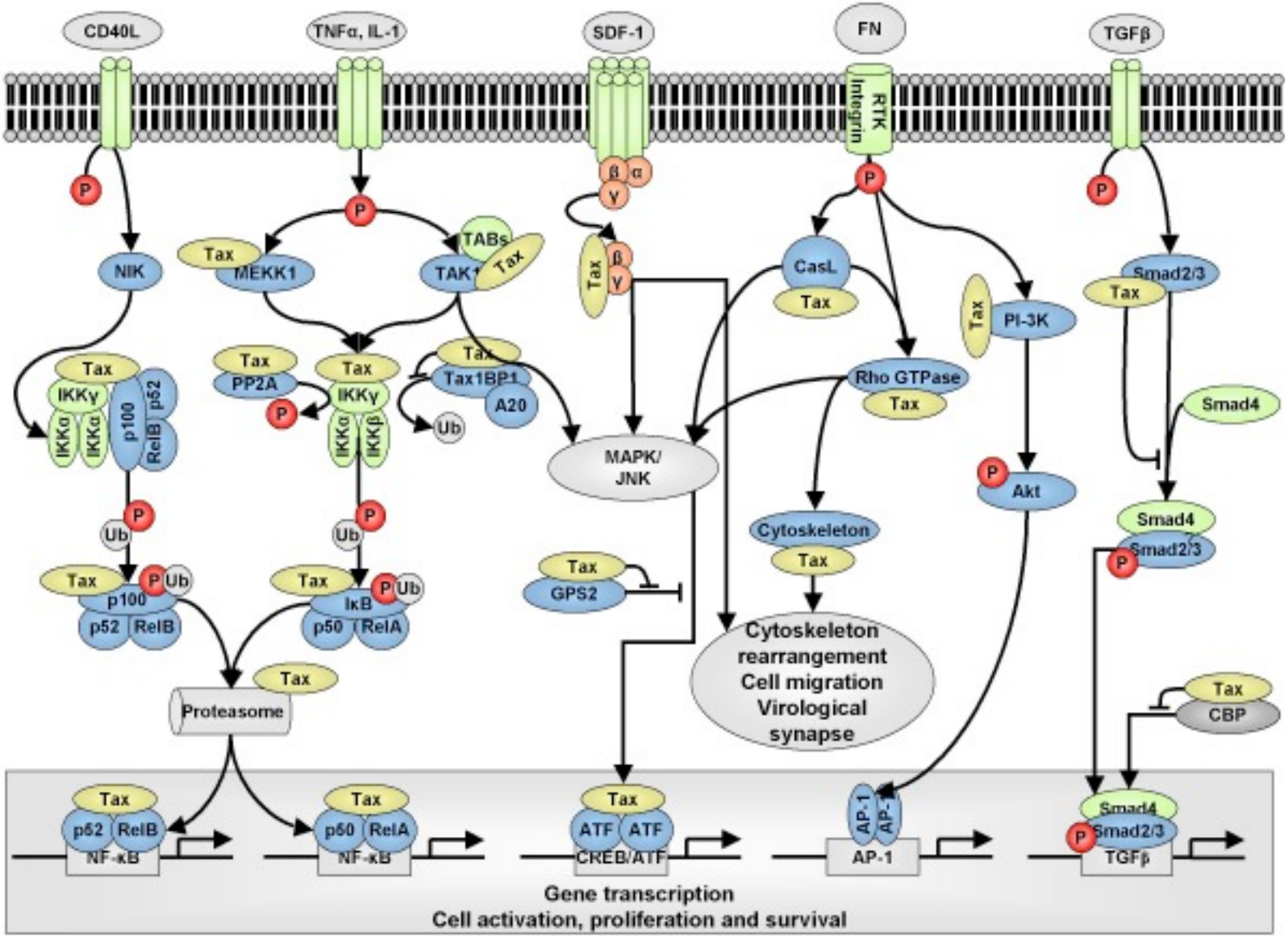


Validation:

PCA

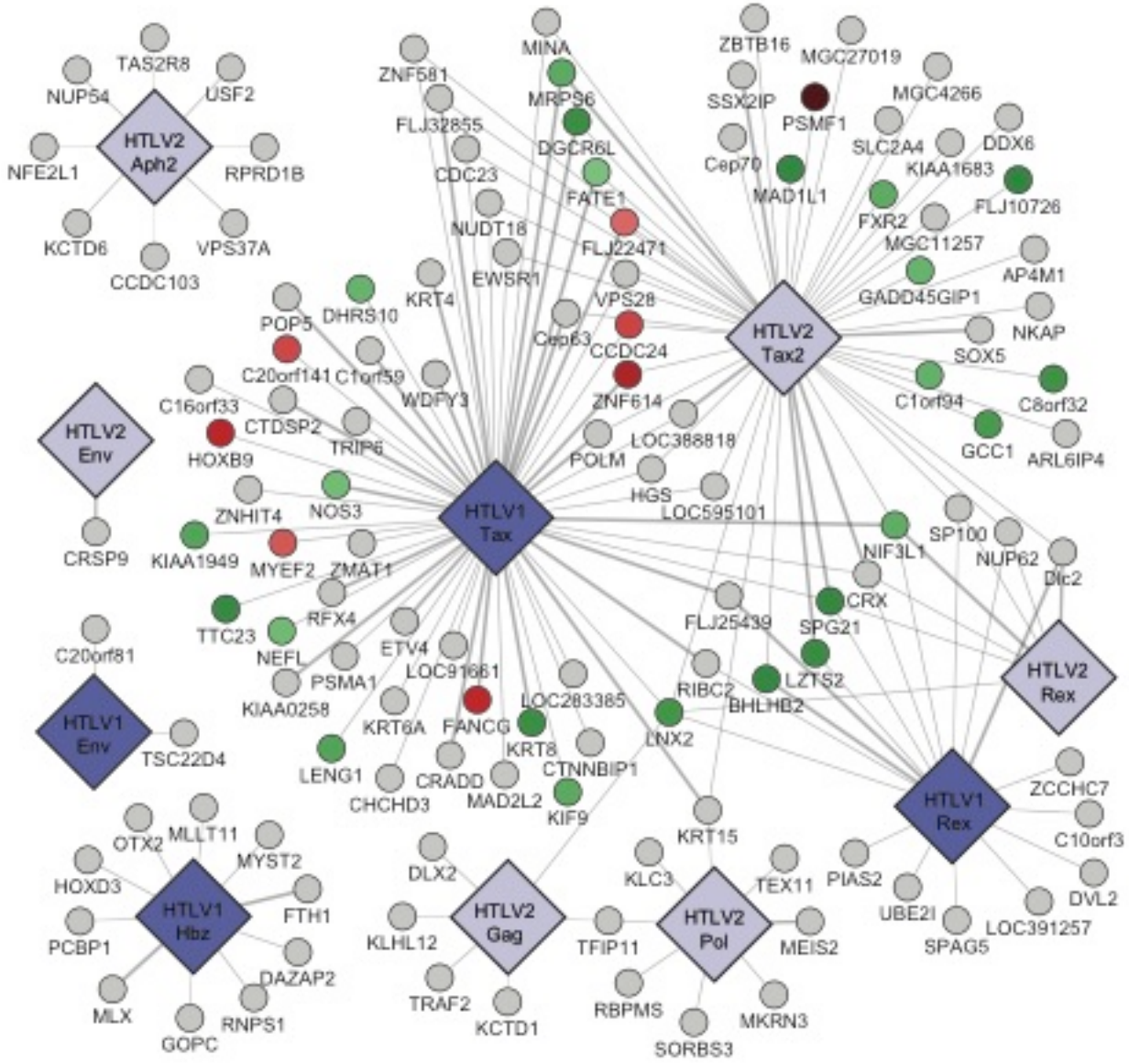
MAPPIT

History



Tax1 Interactome
(Boxus et al. Retrovirology, 2008)

A host – pathogen interactome for HTLV1/2

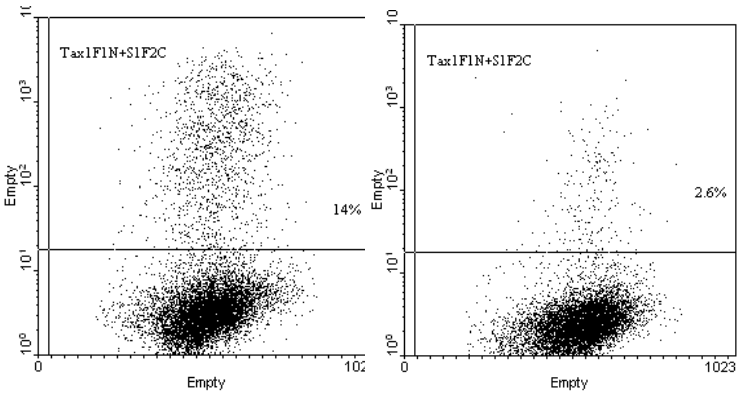


Simonis et al., 2012

Inhibition of protein – protein interactions by small molecules

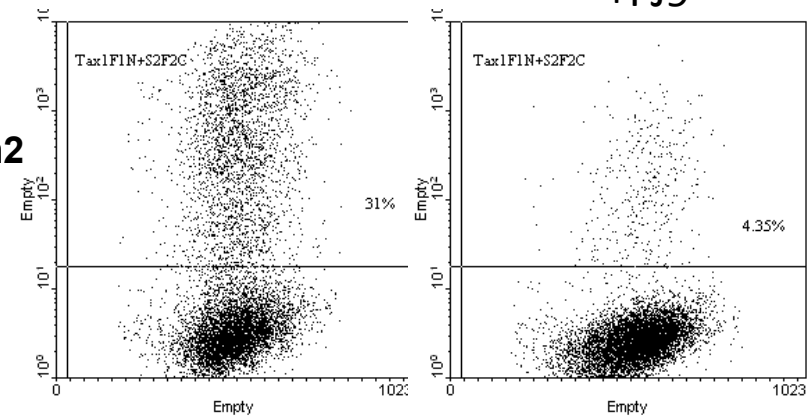
Syntenin1 +Tax1

+FJ9

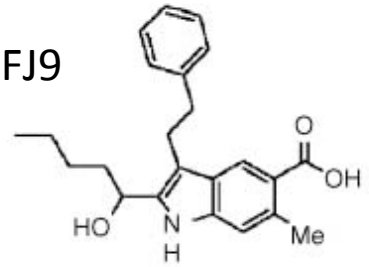


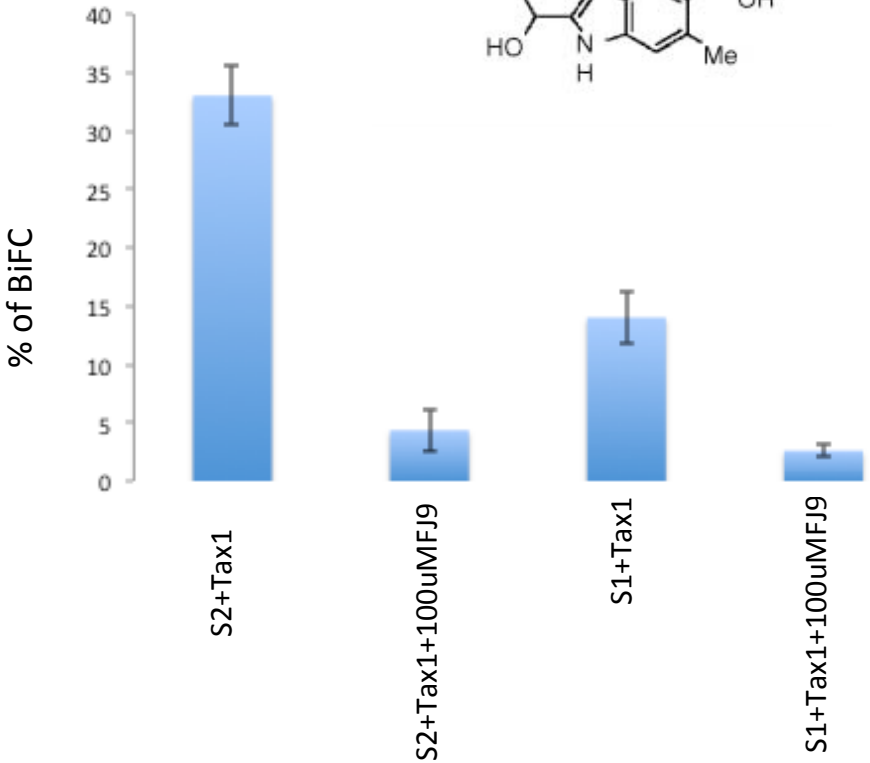
Syntenin2 +Tax1

+FJ9



FJ9

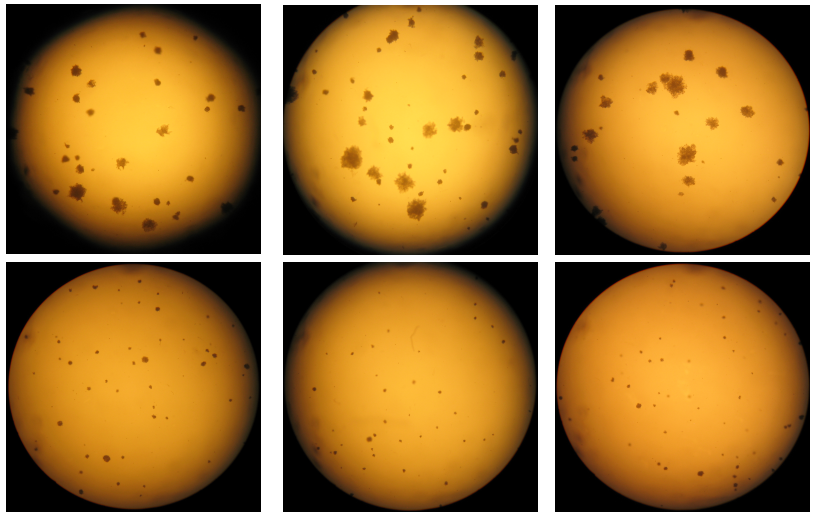




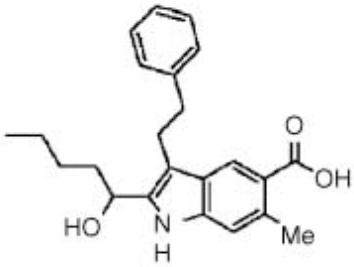
Inhibition of protein – protein and cellular transformation

Rat1-Tax1

Rat1-Tax1 + FJ9



FJ9



Disruption of Tax/ PDZ interaction inhibited Tax transformation as measured by a decrease in size and number of Tax-induced Rat-1 foci.

PDZ proteins involved in Tax1 transformation activity

Models for overall functional organization of the cell

1970s-80s

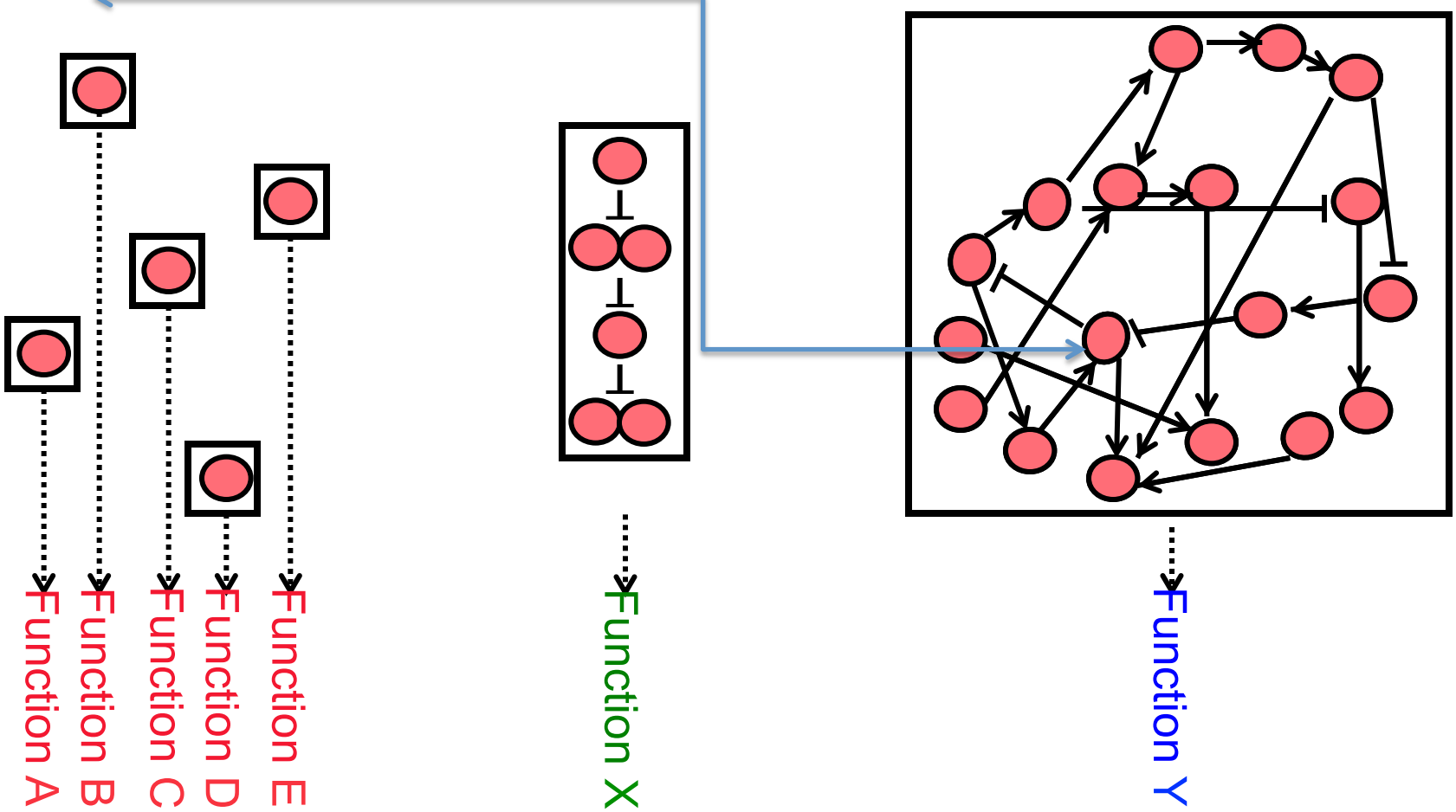
gene-and-protein

1990s

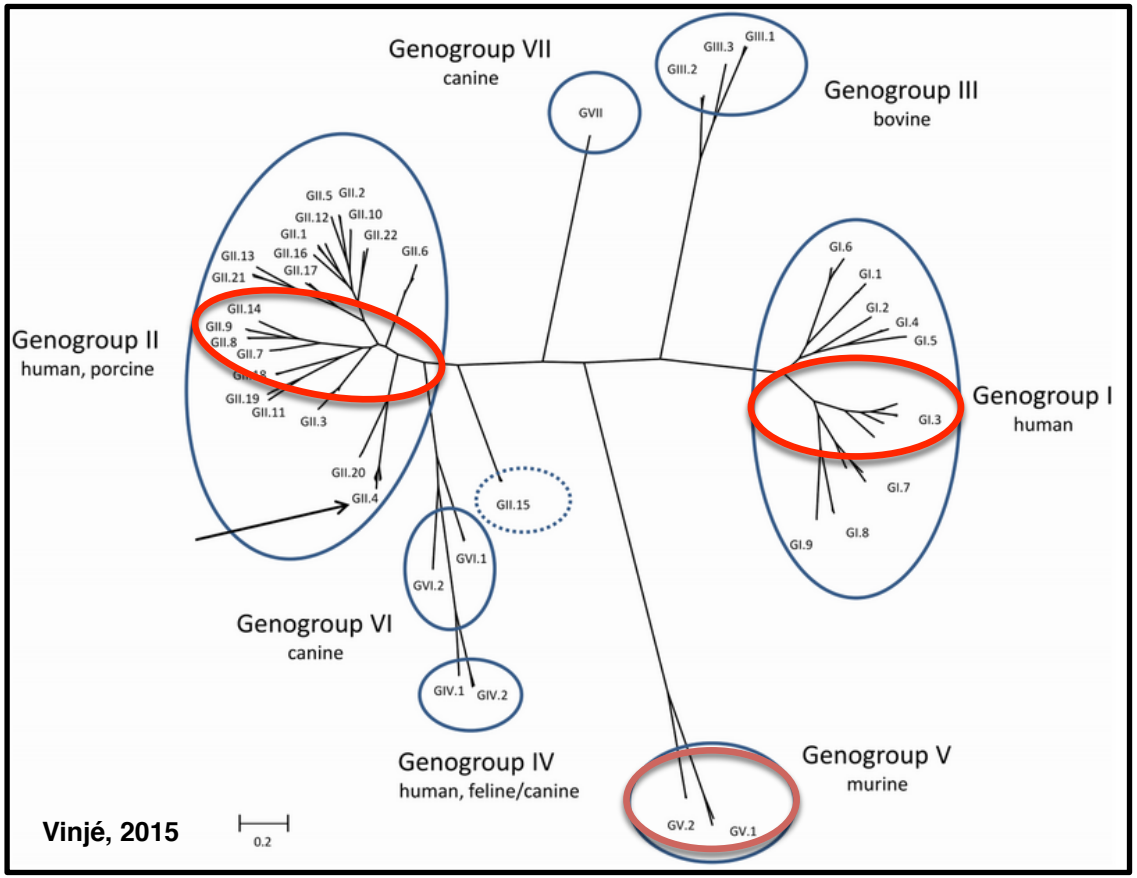
Signaling pathways

2000s

"Interactome" networks



Noroviruses Interactome



GI.1 (ORF2+ORF3)
GI.2
GI.3
GI.4
GI.5
GI.6
GI.7
GI.8
GI.9

GII.1
GII.2
GII.3
GII.4
GII.5
GII.6
GII.7
GII.8
GII.9
GII.10
GII.12
GII.13
GII.14
GII.15
GII.16
GII.17
GII.20
GII.21
GII.22

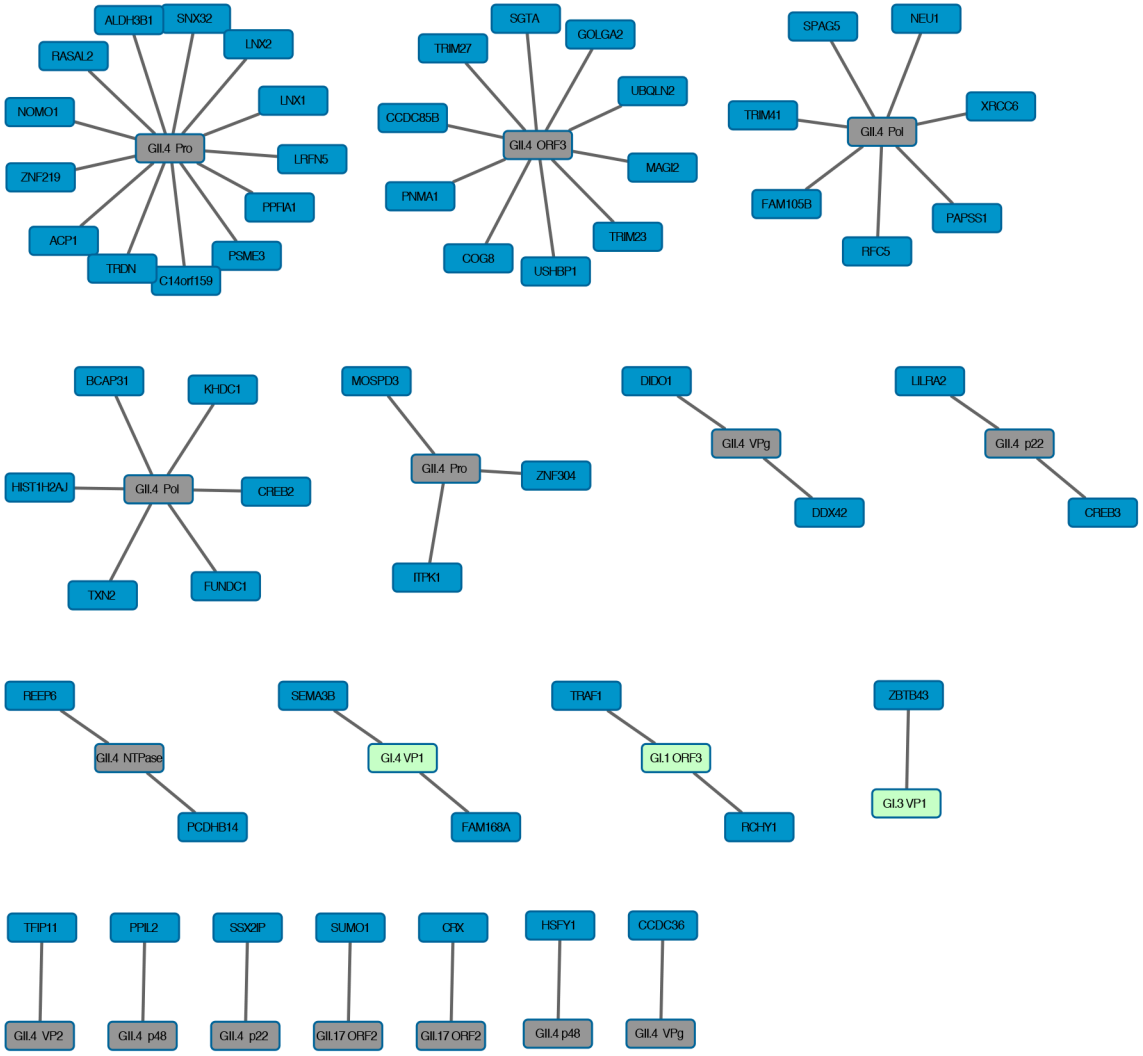
GII.4 1974
GII.4 1987
GII.4 1997
GII.4 2002
GII.4 2002a
GII.4 2004
GII.4 2005
GII.4 2006 (complete)
GII.4 2009
GII.4 2012 (ORF2+ORF3)

MNV1

CW1 (complete; P-domain)
CR6 (complete)
CW3 (complete)

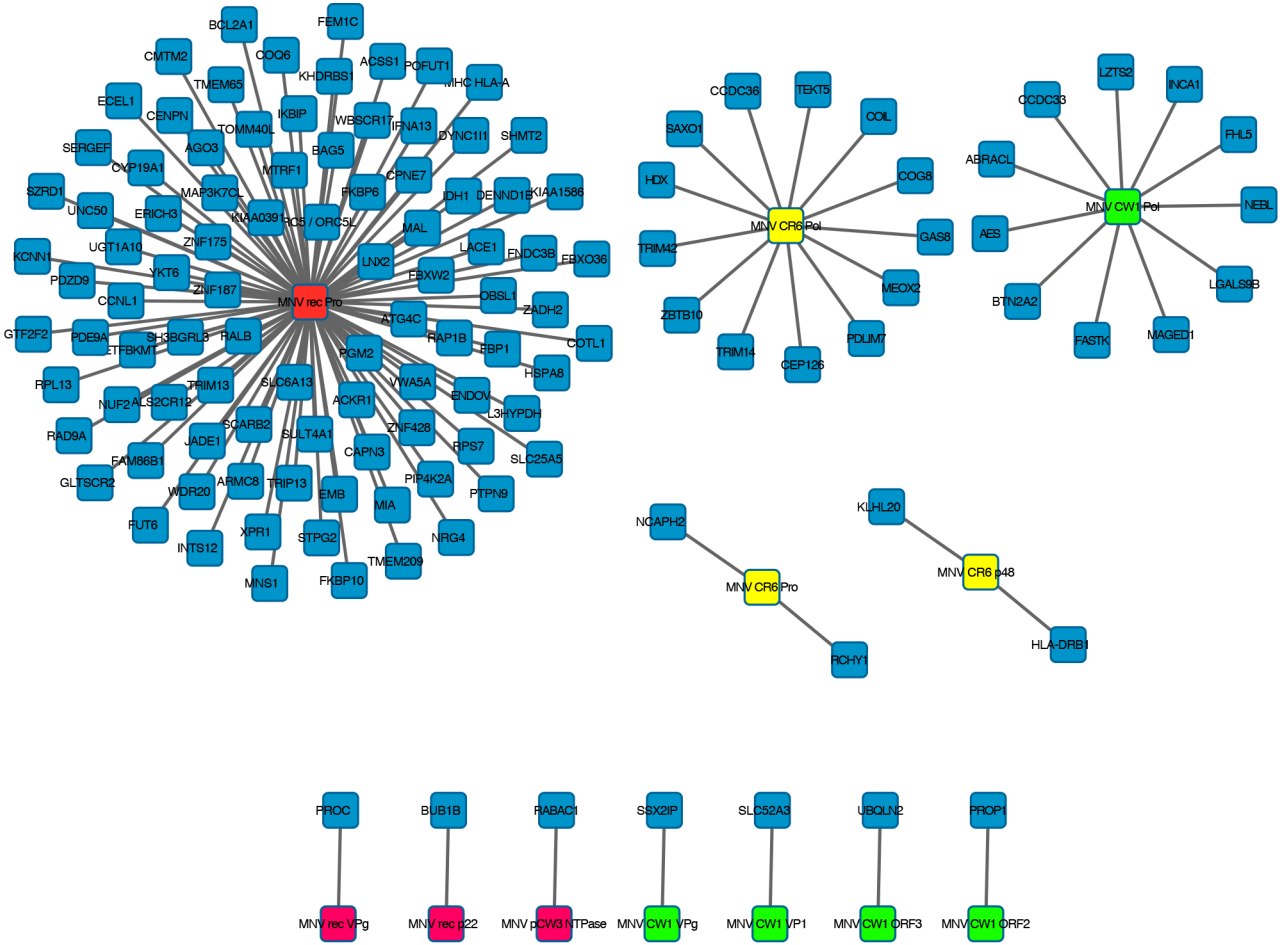
Marco Grodzki, PhD

A human – human noroviruses interactome



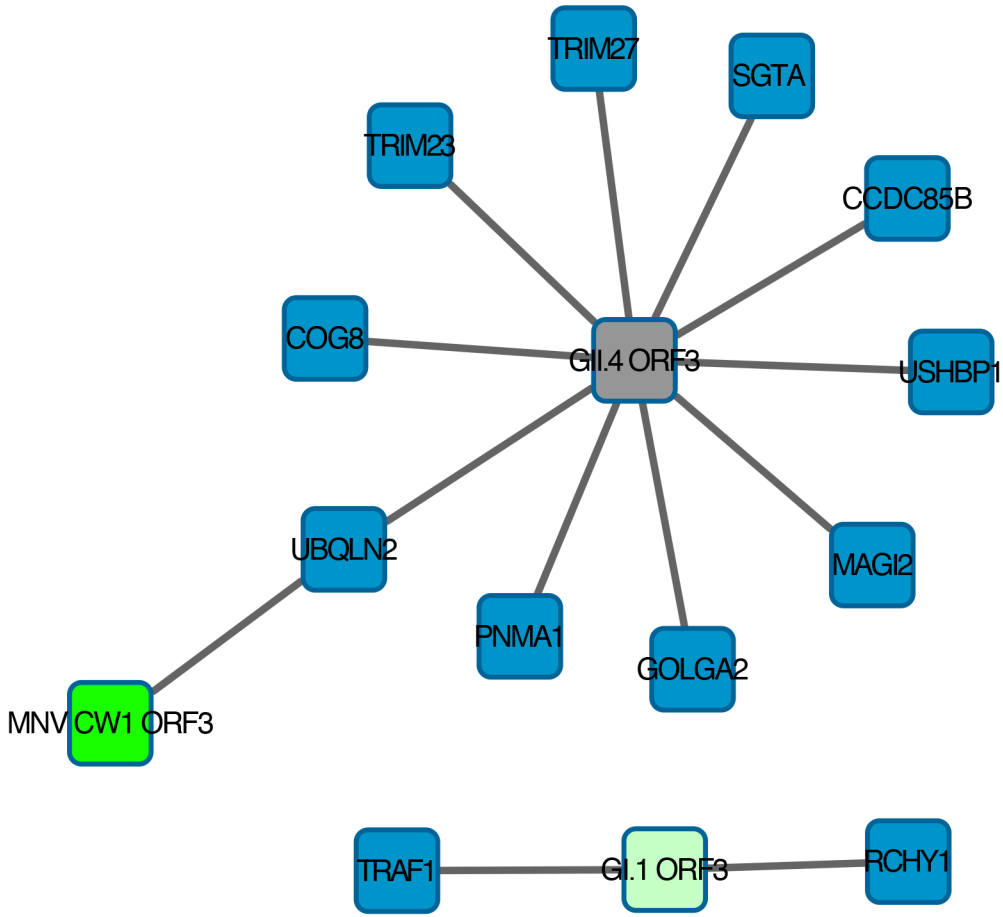
jean-claude.twizere@ulg.ac.be

A human – murine noroviruses interactome



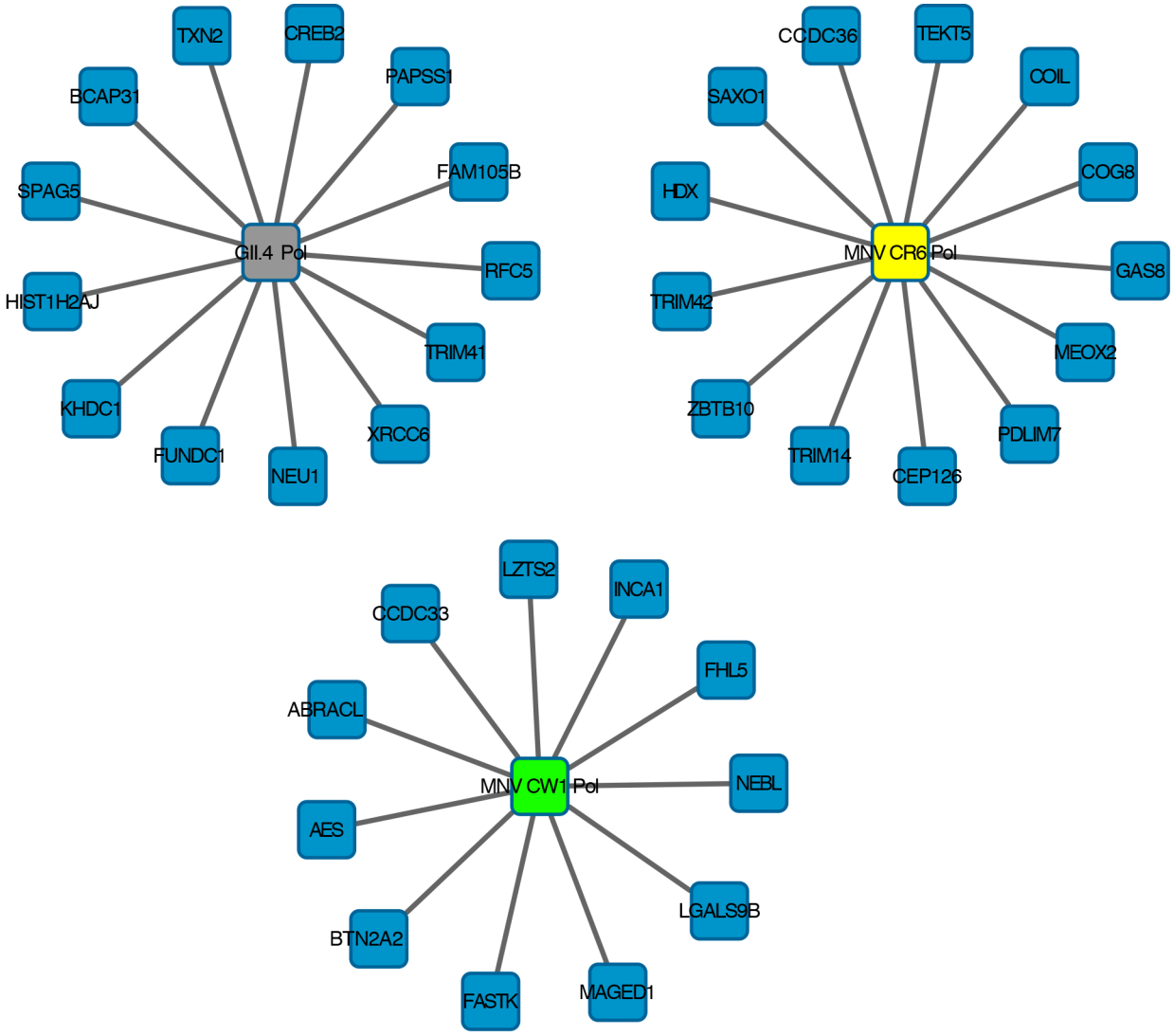
jean-claude.twizere@ulg.ac.be

Comparison HNV – MNV: ORF3



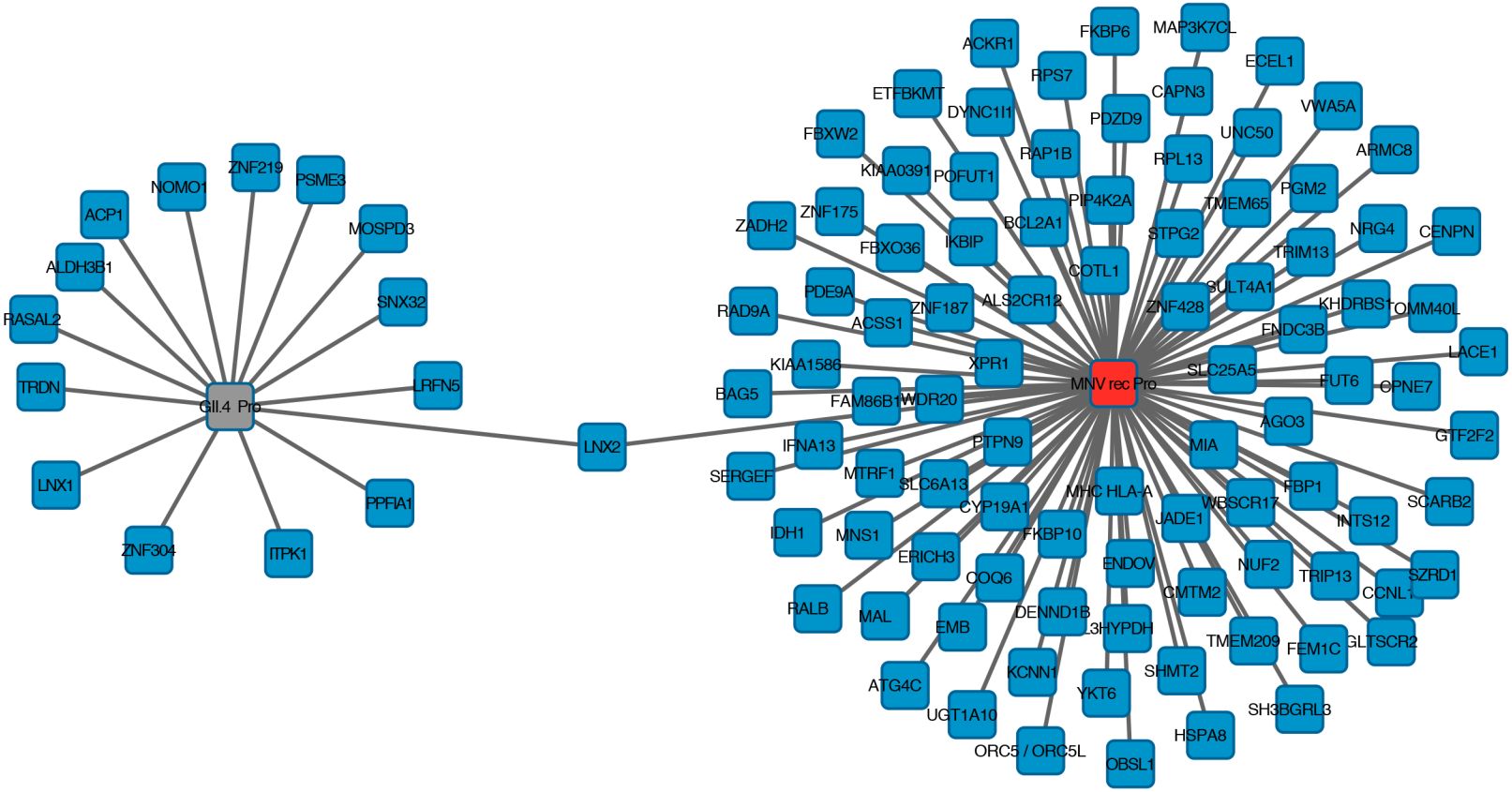
jean-claude.twizere@ulg.ac.be

Comparison HNV – MNV: Pol



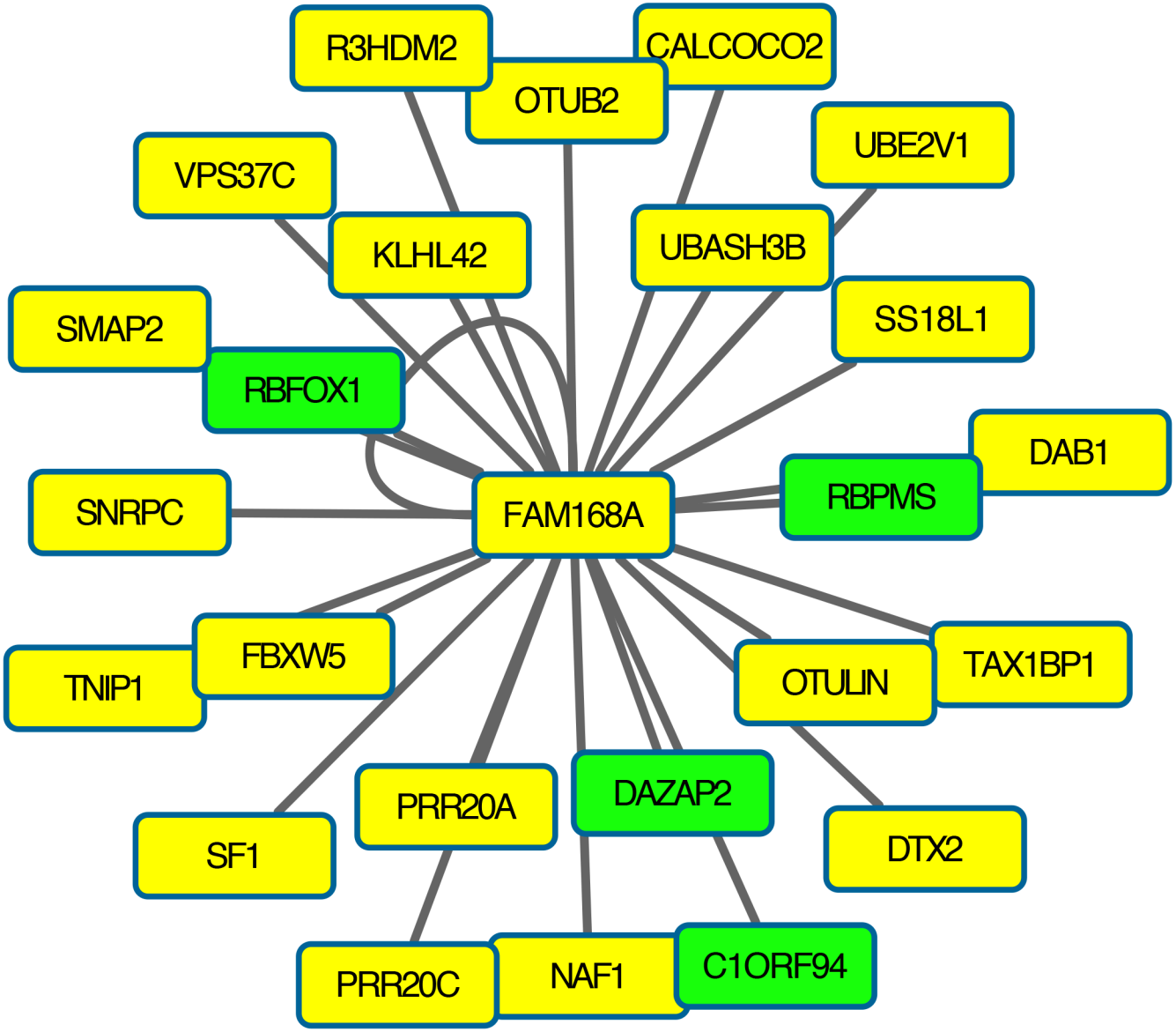
jean-claude.twizere@ulg.ac.be

Comparison HNV – MNV: Pro



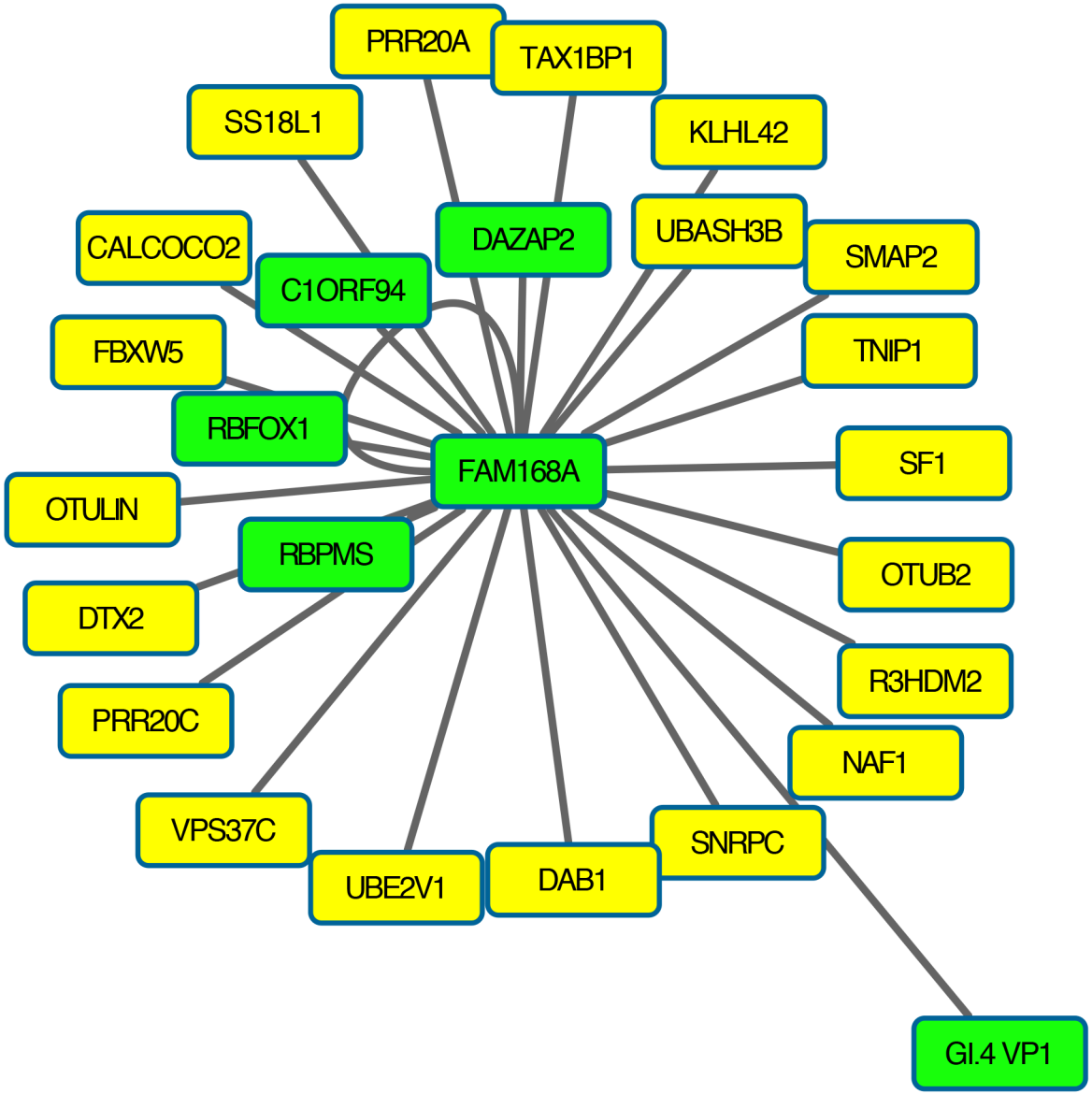
jean-claude.twizere@ulg.ac.be

Targeted hubs in the human interactome: FAM168A (TCRP1)



jean-claude.twizere@ulg.ac.be

VP1 and RNA binding proteins



jean-claude.twizere@ulg.ac.be

Mapping an interactome network

All proteins

All proteins

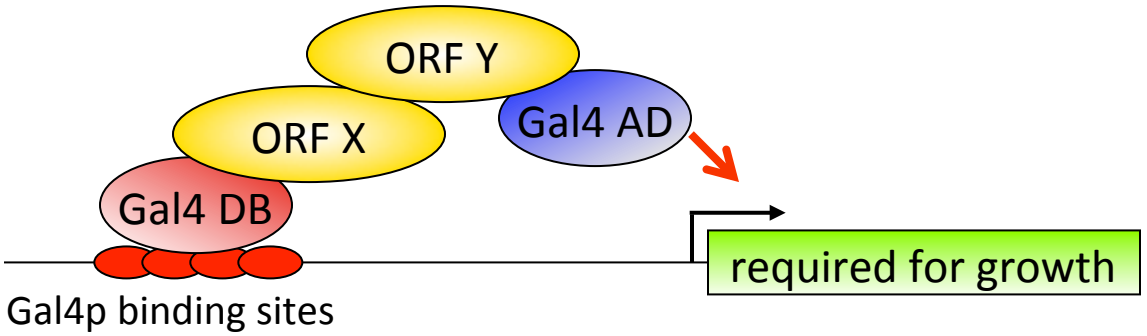
Test

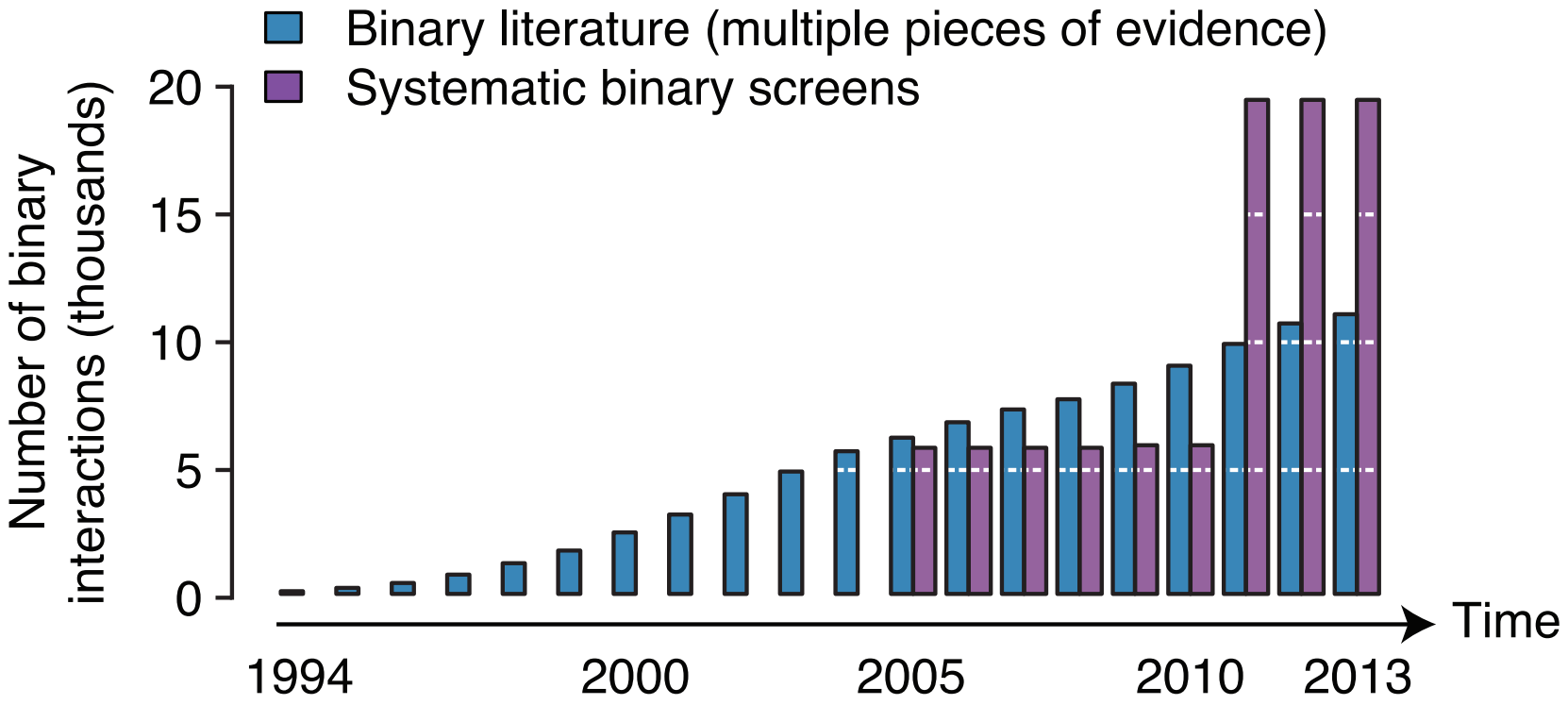
all pairwise combinations

for **possible** physical interactions

Yeast two-hybrid

- Reconstitution of GAL4 transcription factor
- Fusion proteins DB-ORFX and ORFY-AD
- Reporter gene





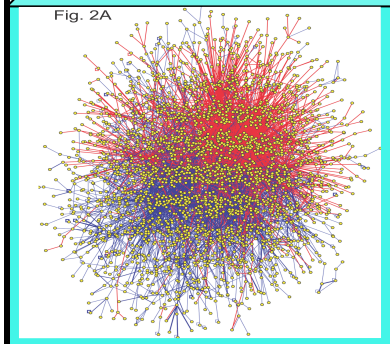
jean-claude.twizere@ulg.ac.be

Rolland et al., Cell. 2014 Nov 20;159(5):1212-26.

Human interactome

Rual et al.; Nature 437, 1173-1178
Stelzl et al.; Cell 122 (6), 957-68

>22,000 proteins

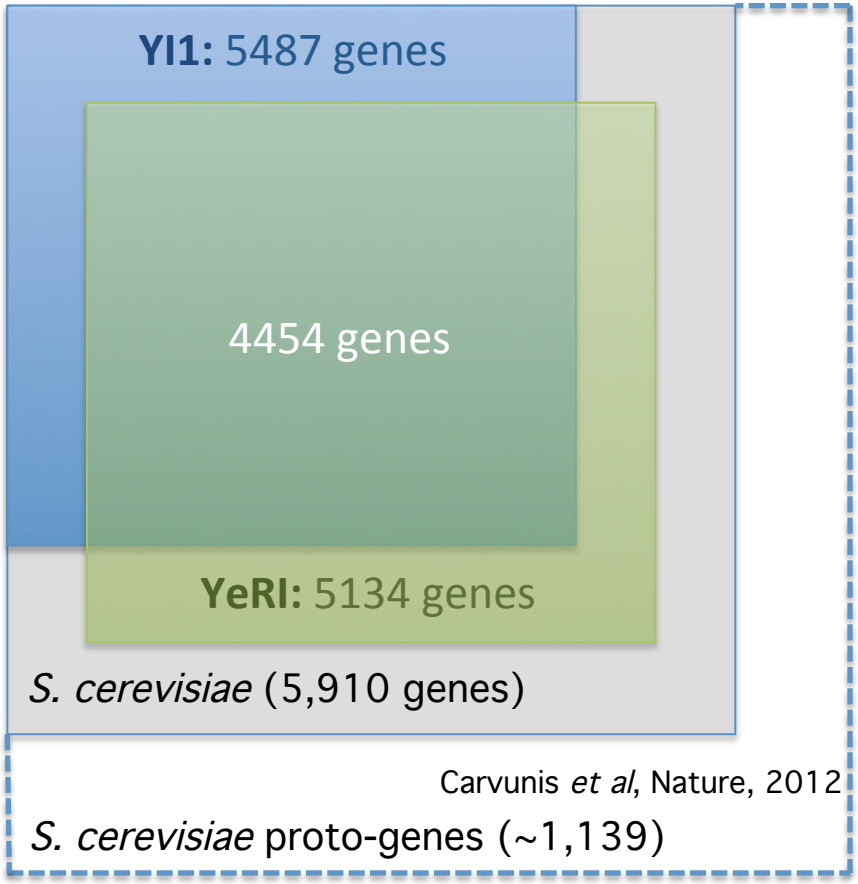


> 5 x 10^8 pairwise combinations

various approaches

>22,000 proteins

Towards completeness of the yeast interactome

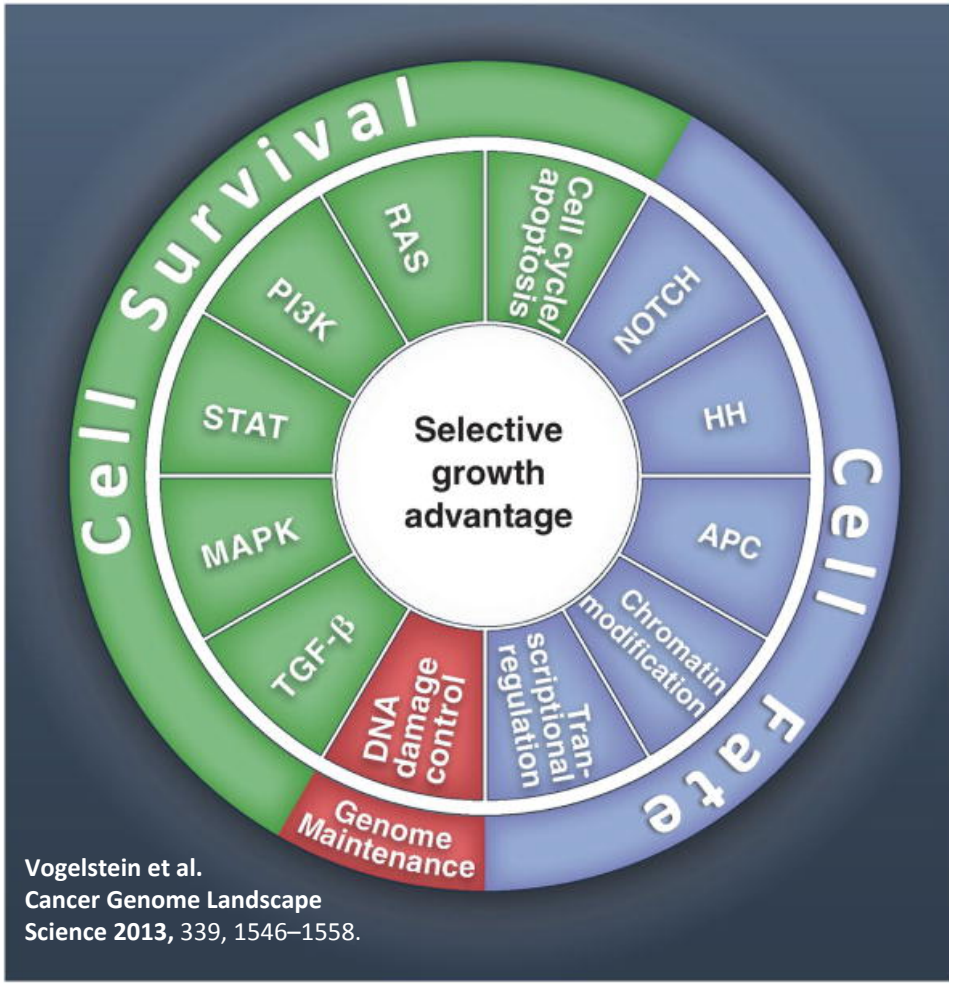


Genomic mutations landscape in cancer

~ 500 cancer census genes

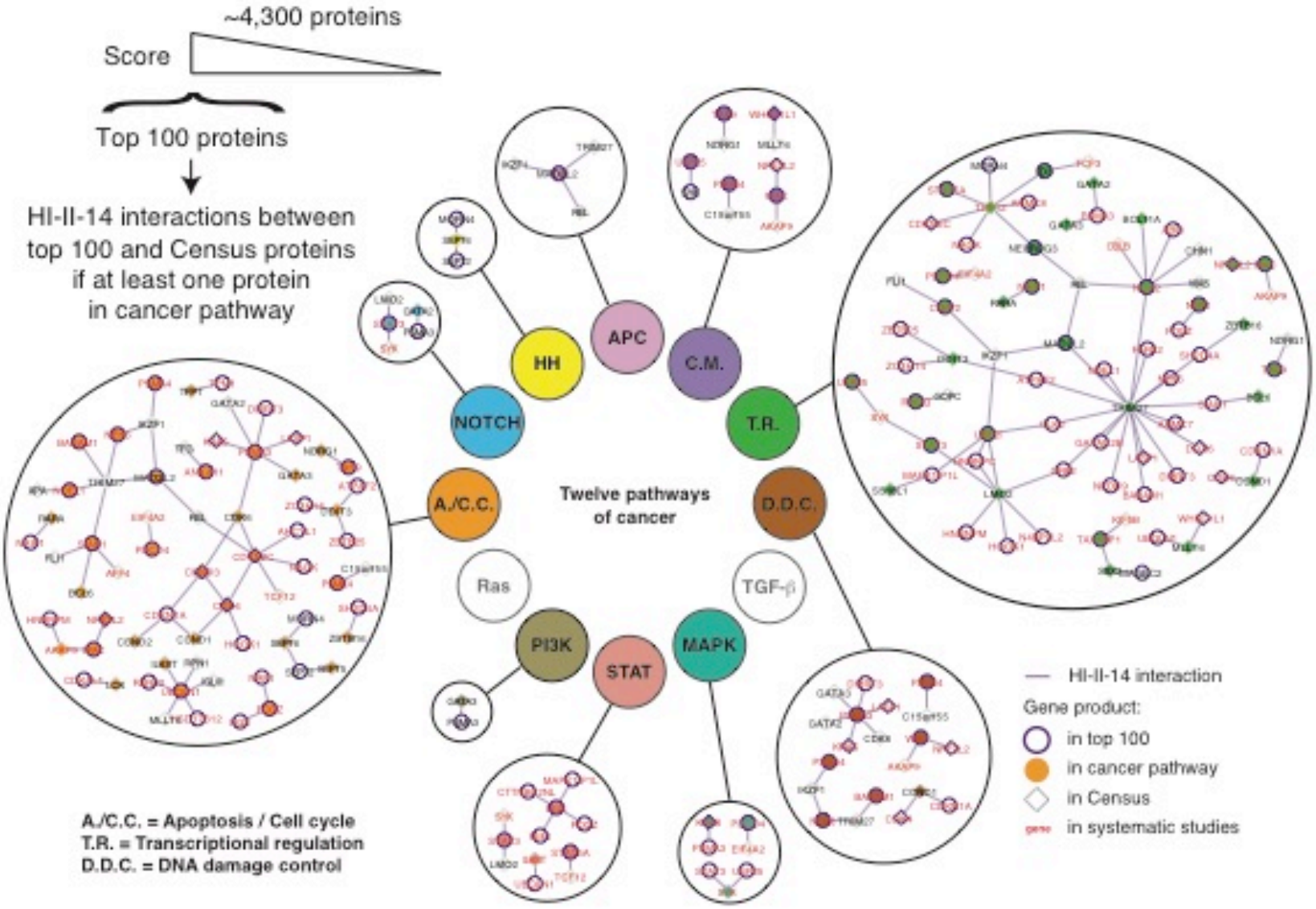
~140 cancer driver genes

Cancer Pathways



Vogelstein et al.
Cancer Genome Landscape
Science 2013, 339, 1546–1558.

Guilt by association partners of known cancer genes



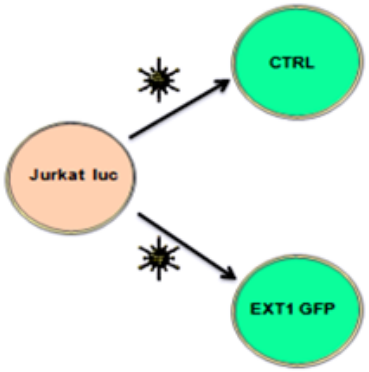
Rolland et al., Cell. 2014 Nov 20;159(5):1212-26.

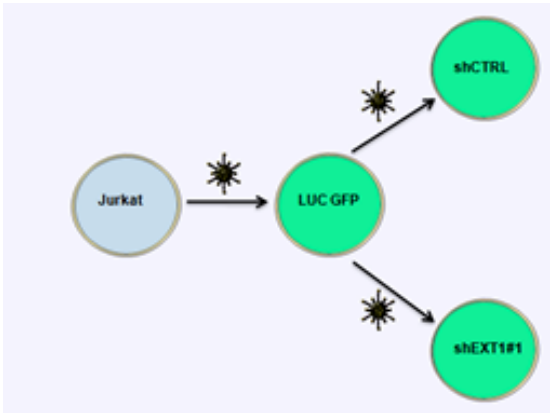
1. The role of EXT1 in T-ALL

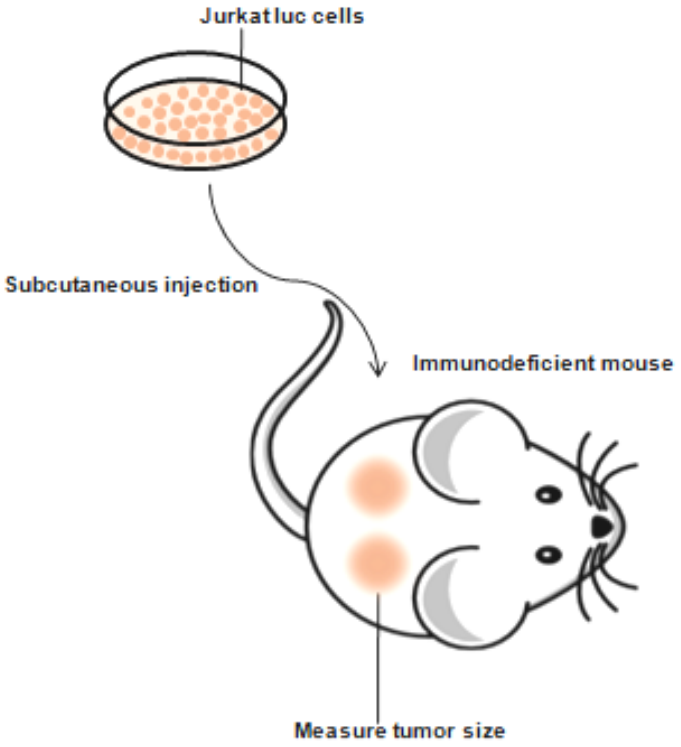
Silencing/over-expression of EXT1 in a T-ALL in *vivo* model

Tumor xenograft experiment

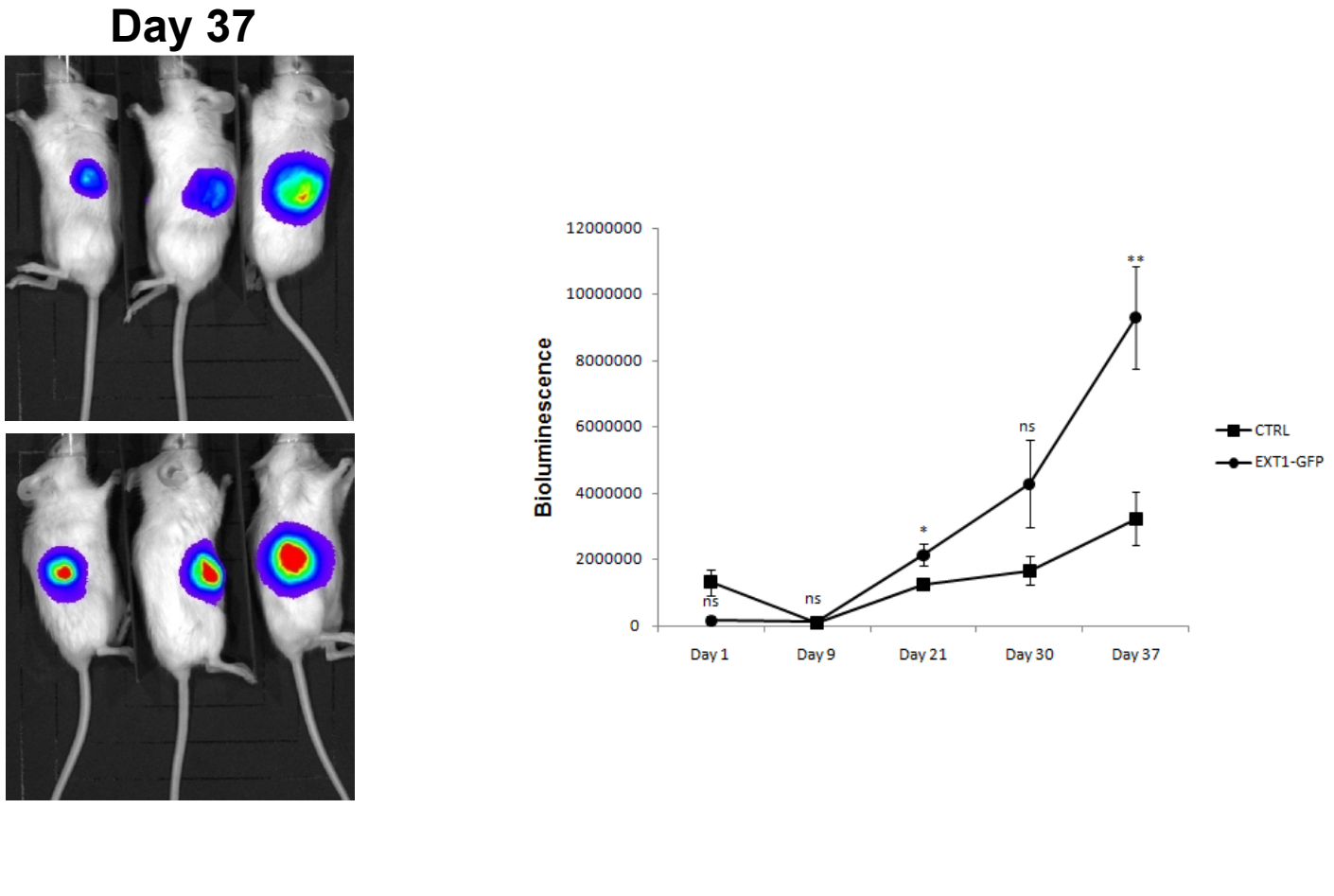
Based on bioluminescence imaging (BLI) with luciferase reporter







The role of EXT1 in T-ALL

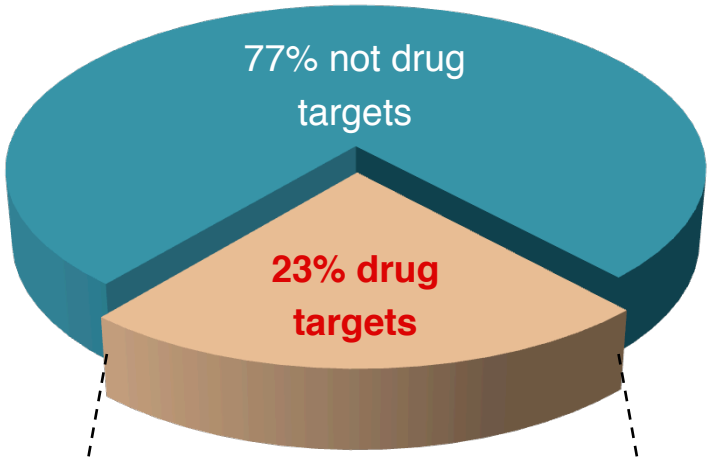


Injection of Jurkat over-expressing EXT1 in NOD-SCID mice resulted in a significant increase of the leukemic burden

Drug discovery is facing a crisis

Functional-based

Disease-associated proteins
6,523*



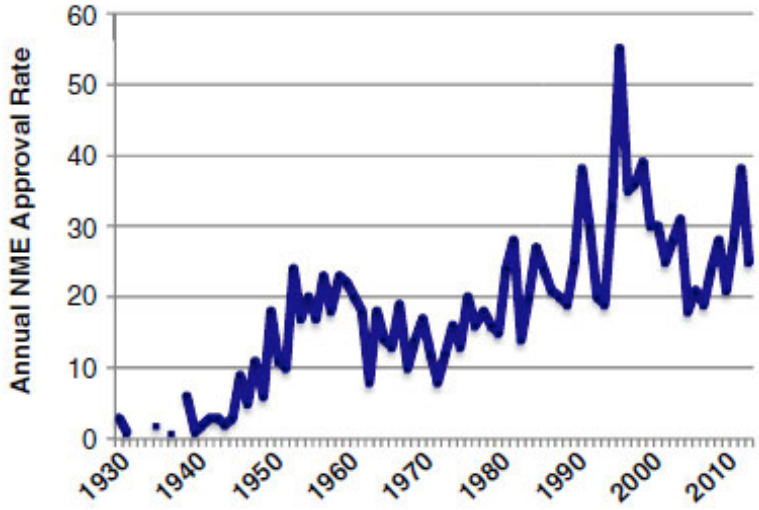
95% = 5 protein families**
Enzymes, Transporters, GPCRs, Ion channels, Other receptors

5% = Others

Databases: HGMD*, IUPHAR**

Success story

FDA-approved **drugs per year**



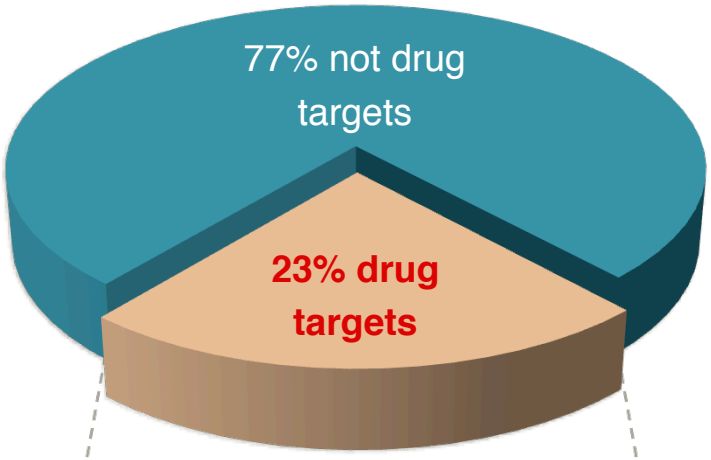
Stagnation !

Kinch *et al*, *Drug Discov Today* (2014)

The potential of protein-protein interactions (PPIs)

Functional-based

Disease-associated proteins
6,523*



95% = 5 protein families**
Enzymes, Transporters, GPCRs, Ion channels, Other receptors

5% = Others

vs.

PPI-based

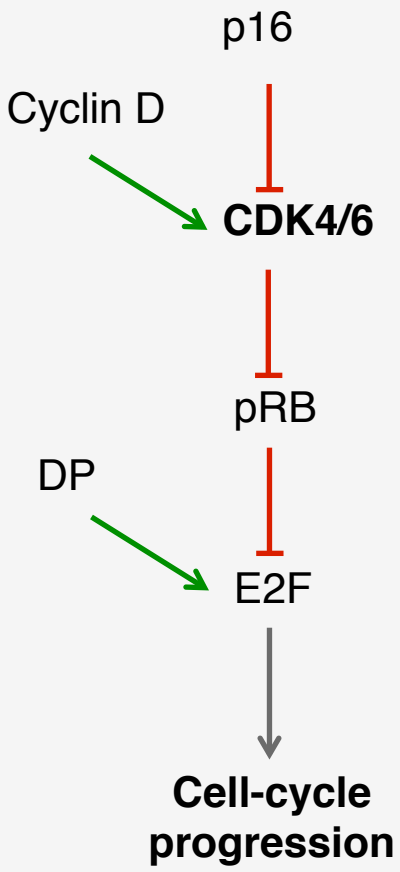
Disease-associated proteins
6,523*

Expansion of the landscape of drug targets

Databases: HGMD*, IUPHAR**, Mentha***

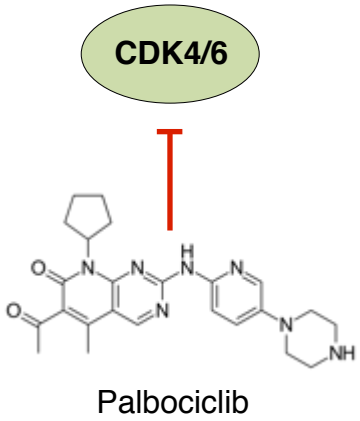
The PPI-based approach in practice

pRB pathway

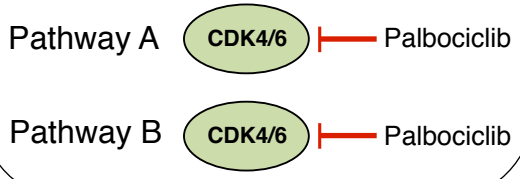


Functional-based

One target



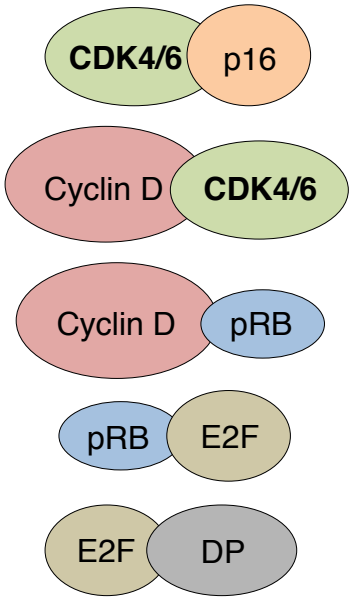
Several off target effects



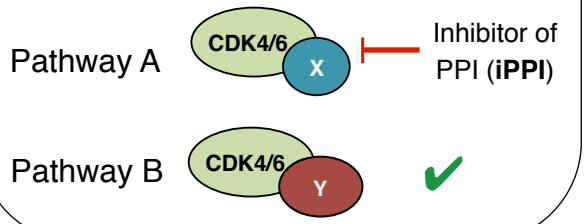
vs.

PPI-based

Several targets

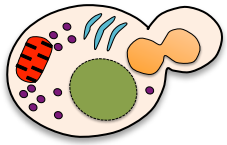


Higher selectivity (?)

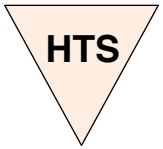


How to develop an alternative PPI-based approach?

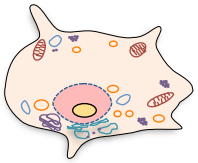
We need…



an **experimental system**

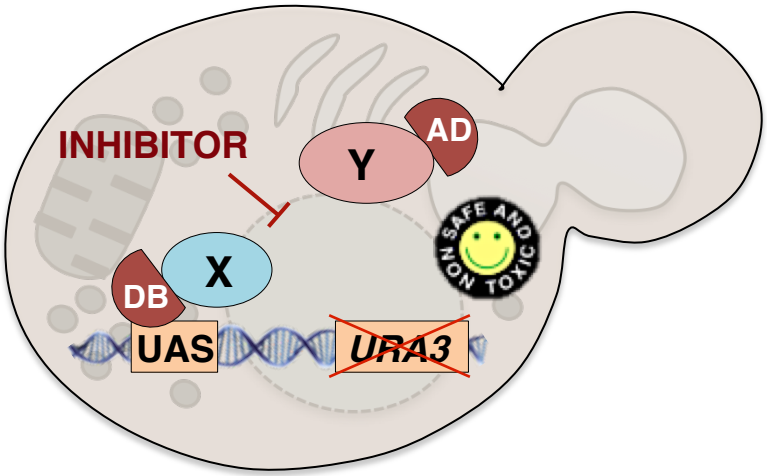


that is **scalable** for systematic/high-throughput screening (**HTS**), and



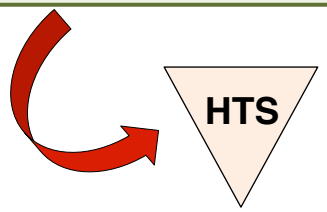
for which powerful **validation assays** are available

The reverse yeast two-hybrid (RY2H) assay

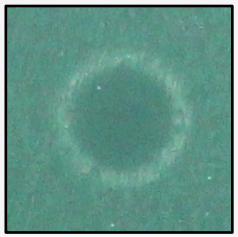


Positive selection
(growth) of **iPPIs**

- ✓ **Cell permeability**
- ✓ **Cytotoxicity**
- ✓ **POOLING of PPIs**



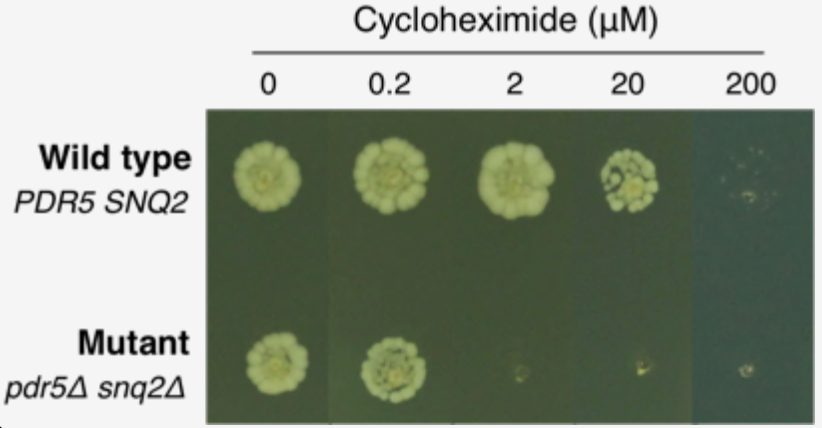
Detection of iPPIs



PPI inhibition
=
ring of growing cells

e.g. AD-**FKBP12**/DB-**RIC**
disrupted by **FK506**

Drug sensitive strain



Julien Olivet, Hideki Endoh & Marc Vidal

Pooling of PPIs for ultra HTS via RY2H

HTS

- Test PPI-based approach: **1,700 PPIs encompassing pRB & BRCA1** pathways
- **Systematic assessment** of pRB & BRCA1 pathways:

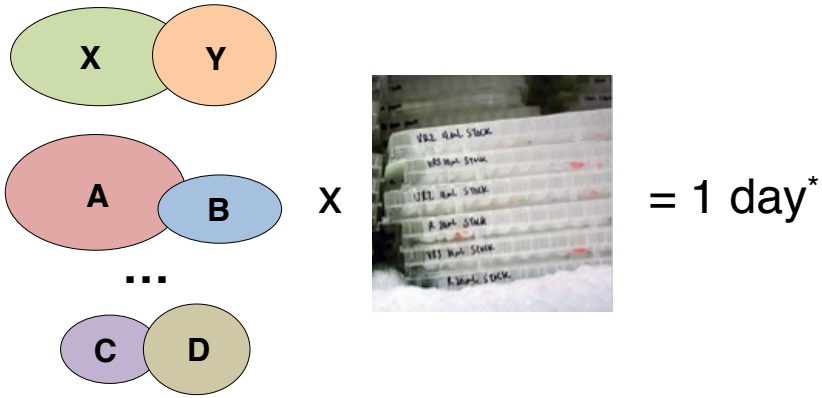
Individual PPIs screened

One PPI **10,000 drugs**

For 1,700 PPIs: **~5 YEARS**

Pools of PPIs screened

100 PPIs **10,000 drugs**



For 1,700 PPIs: **20 DAYS**

*Robotics set up at the drug screening platform of Harvard Medical School

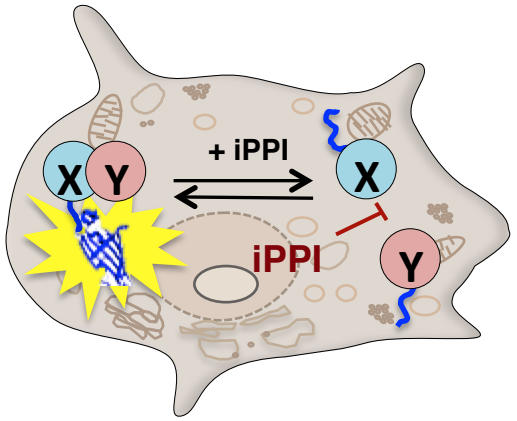
Vidal & Endoh, *Trends Biotechnol* (1999)

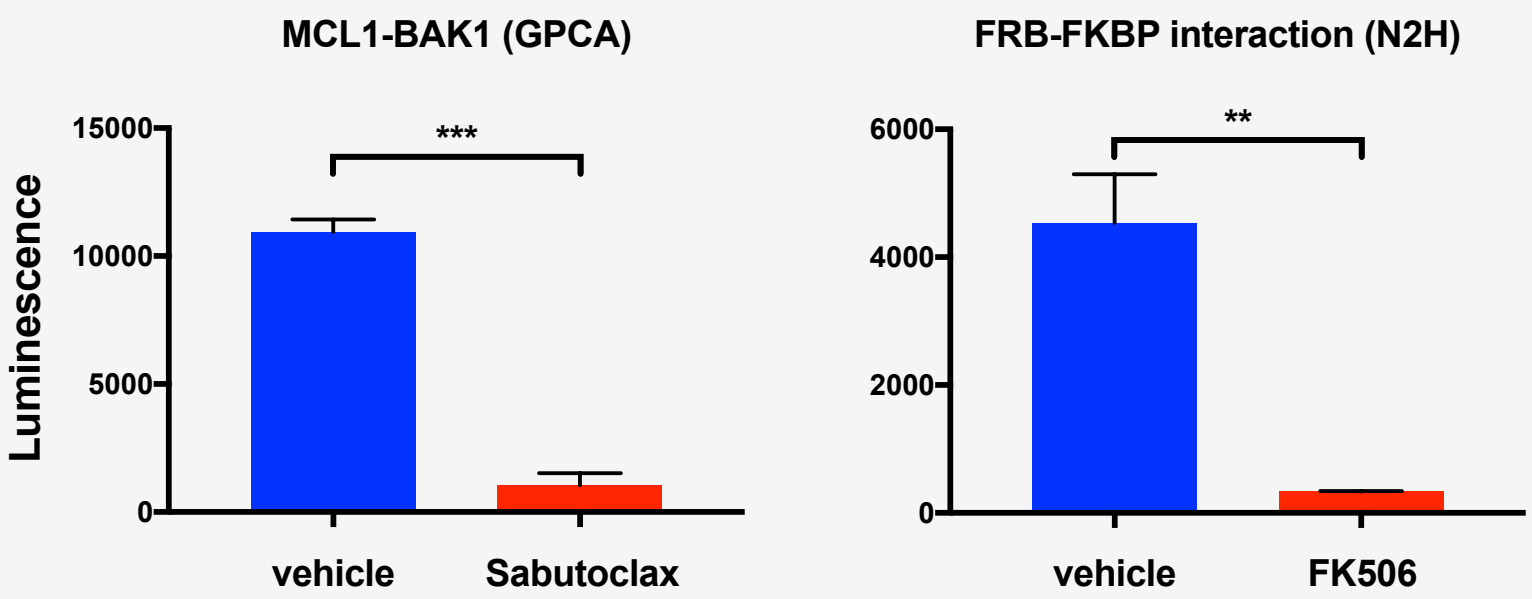
Validations of primary iPPIs from screening

Mammalian cell-based binary PPI assays

- *G. princeps* luciferase-based Protein Complementation Assay (**GPCA**)*
- Nanoluciferase Two Hybrid (**N2H**) assay

Choi, **Olivet** *et al*, in preparation (2017)





Cassonnet *et al*, *Nat Methods* (2011)*

Applications of interactome mapping

- Organisms Interactome mapping
- Novel disease-related genes
- Host-Pathogens interactomes
- Novel therapies identification

jean-claude.twizere@ulg.ac.be